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THE MIGRATION OF BACTERIUM TUMEFACIENS IN THE TISSUE OF TOMATO PLANTS[1]

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LITERATURE REVIEW

The literature on the crown gall of plants is extensive and in most of its phases is well known. References to the migration of the causal organism, *Bacterium tumefaciens*, Sm. and Town., (*Pseudomonas tumefaciens*, Sm. and Town., *Phytomonas tumefaciens*, S. A. B.) through the tissues of the host are to be found mainly in the reports of investigations by Smith, by Riker, and by Robinson and Walkden.

Smith and his associates (11), in the extensive earlier literature on *B. tumefaciens*, regarded the organism as an intracellular parasite. Their conception of the migration was that the infected tissue proliferated in a manner similar to that of cancerous animal tissue, pushing its way into and through healthy tissue, carrying the bacteria along within the cells. They state, "It is probable that the parasite in its migration from one part of the plant to another does not make free use either of the vessels or of the intercellular spaces, at least, we have not been able to find it in them. Rather, we think it is imprisoned within the specially stimulated and rapidly dividing cells and is by the growth of these cells carried along." Again, Smith (8) states, referring to the crown gall organism, "From what I have seen, I believe it occurs only inside the parenchyma cells, stimulating them to divide and passing on from mother cell to daughter cell in this manner." Smith (9) points out many similarities of the crown gall disease to the cancers of animals, especially emphasizing the connections between the "primary" and "secondary tumors." These connections were termed "tumor strands" and were regarded as proliferating tissue of the "primary tumor." Referring to this situation and regarding the

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migration of the organism, he states, "The organism causing this tumor occurs only inside of certain of the proliferating cells. When the cell divides, the organism is carried over into the daughter cells, in at least a part of which it multiplies."

Cook (1), in a recent report of an investigation of the early stages of crown gall, accepts the conception of Smith regarding the occurrence of "tumor strands" and "cancer tissue." Levine (2), investigating crown gall with special reference to its resemblance to animal cancers, does not accept Smith's view on "tumor strands" and "secondary tumors" in the crown gall disease. He says, "In no case have I found a growth of crown gall tissue attributable to an inoculation at some distant point." Levine makes no statements as to the methods of migration of the organism. Riker (4, 5, 6) and Robinson and Walkden (7) have shown that the bacteria are consistently located in the intercellular spaces and not within the cells of the host as Smith supposed.

Recently two distinct conceptions of the actual manner in which the bacteria migrate through the host tissues have been added to the earlier hypothesis proposed by Smith. Riker (4, 5, 6), as one of the results of extensive morphological and cytological investigations of crown gall on tomato which emphasize the relation of the causal organism to its host tissue, has reported his observations on the occurrence of *B. tumefaciens* in plant tissue and gives something of his conception of the manner of its migration. He observed the presence of a water-soaked area surrounding the puncture after inoculation. His conclusion was "that this darkened area, which tended to be parallel to the long axis of the plant, was caused by the occupancy of the intercellular spaces by liquid." He stated, upon further investigation, that the resulting tumor or gall at first corresponded in size and shape to this water soaked area and "closely coincided with it in outline." He conducted experiments the purpose of which was to increase the area having water in the intercellular spaces. Bruising and freezing the portions of the stem followed by inoculation with *B. tumefaciens* resulted in the formation of galls over an extended area. Riker implies that the migration of the bacteria is through the fluid which has been released into the intercellular spaces by the puncture of the needle. He says, "These experiments indicated that the bacteria could travel several centimeters at least if they were given a continuous channel of fluid through which they might pass." Again referring to the presence of the bacteria in relation to gall formation, he says (4), "The observed facts seemed rather to indicate the possibility that the organism began its activities in the liquid which occupied the intercellular spaces around the puncture." Riker (5), after referring to the inoculation of a stem by

puncture, states, "It seems unquestionable that the bacteria once inside such a wound, would migrate to the limits of the avenue provided by the flooded intercellular spaces." Riker again (4) states, "In order to understand more clearly the relations of the organism to the liquid in the intercellular spaces, it seemed advisable to make observations on its motility both in water and in expressed plant juice." Therefore, measuring the rate of motion of *B. tumefaciens*, he found (4, page 123) that motile bacteria in sterile distilled water or expressed tomato sap move approximately at the rate of 1 mm. per minute. After giving the results of these measurements, he states, "This gives an easy explanation of how the organisms might reach the limits of the region flooded with the liquid which was released by the puncture." Nowhere does Riker report any direct observation of the manner or method of the migration of *B. tumefaciens*, but from his statements one is led to infer that he conceives of the migration of the bacteria as due to the motility of the individual organism in (or through) the water or cell sap released into the intercellular spaces by the puncture of the inoculating needle.

Riker (4) also conducted some experiments designed to determine whether the bacteria had to be inserted into a wound or if they could enter a wound from the external surface of the stem. He demonstrated in 50 instances, properly checked, that they enter a wound from the outside of the stem and produce a gall in 100 per cent of the trials. He says, "The forces which govern this entry of the organism into the tissue are not definitely understood. The bacteria might conceivably be influenced by any or all of such factors as the collapse of drying tissue, negative pressure, sap rise, and motility with or without a chemotactic stimulus." In a second set of experiments he obtained galls in 50 per cent of the cases in which numbers of *B. tumefaciens* were applied to the surface of the stem at the needle punctures six days after they had been made. That cell sap released into intercellular spaces by a needle puncture might be resorbed in a period of six days, is apparently not considered a possibility.

Riker (4) also reports the passage of *B. tumefaciens* through the vessels of the xylem in the tomato stem.

In a series of experiments designed to determine the effects of inoculating water-soaked plant tissues with *B. tumefaciens*, Levine (2) found that the gall was produced only at the point of entry of the inoculum, and not throughout the entire water-soaked area as reported by Riker. Levine concludes that the size of the gall is related to the age of the tissue, larger galls developing in younger parts of the stem. Levine's results on this point cast doubt on the correctness of Riker's conclusion as to the importance of the water-soaked area in the migration of the bacteria.

Robinson and Walkden (7), in a critical report of their investigations of crown gall, express an entirely different conception of the migration of the causal organism. They studied the disease in *Nicotiana affinis* and in *Chrysanthemum frutescens*. Their reference to the migration of the bacteria is rather incidental to the main topic of their report, which deals primarily with the location and distribution of the bacteria in the tissues and their relationship to the "secondary galls" and the so-called "tumor strands." Their conception of the migration is that the bacteria advance through the intercellular spaces as zoogloeae. They state, "Pl. VI, Fig. 22, shows, more highly magnified, the zoogloeal strand of *B. tumefaciens* seen advancing through a large, longitudinal, intercellular space in the pith of *Nicotiana*." Again they state, "The bacteria often associated as zoogloea-like strands have, . . . been traced for shorter or longer distances from the point of inoculation. The course of this bacterial extension is by way of intercellular spaces and protoxylem." In their summary these investigators state, "Both in *Chrysanthemum frutescens* and in *Nicotiana affinis* we have definitely demonstrated, by staining, zoogloeal strands of *B. tumefaciens* intruding along intercellular spaces and protoxylem vessels, forming centers for pathological disturbances and gall production along the tract."

These recent investigators, Riker (4, 5, 6), Robinson and Walkden (7), and Levine (2) all disagree with Smith (8, 9, 10, 11) as to the nature of the "tumor strands" and "secondary tumors" in the crown gall disease and consequently as to the manner of migration of the bacteria. Riker concluded that the tissue of the tumor strand arises as a result of a stimulation to division of the cells along the path of migration of the organism and not as proliferating strands of tumor tissue as Smith stated. Robinson and Walkden found secondary tumors, but regarded them as due to the migration of the bacteria through the intercellular spaces, especially through the pith and not as due to a proliferating tumor strand.

Robinson and Walkden agree with Riker as to the region but not as to the exact manner of migration. In contrast to the earlier conception of Smith that *B. tumefaciens* is an intracellular parasite, Robinson and Walkden as well as Riker have agreed that the migration is through the intercellular spaces and the xylem vessels. Riker, however, implies that the migration is due to the motility of the individual organism through water or released cell sap in the intercellular spaces, while Robinson and Walkden regard the migration as an intrusion of organized masses of the bacteria or zoogloeae into the intercellular spaces and even the vessels.

Smith (10), referring to the presence of the bacteria within the cells, in one of his last contributions adds a footnote as follows: "Recently Riker

in the United States and Robinson and Walkden in England have denied this, maintaining that the organism is always between the cells and the subject is still in dispute."

INVESTIGATION

Materials and Methods[2]

In this investigation I studied particularly the method of migration and the rate of migration of *B. tumefaciens* through the host tissues and I am confining this paper to these points. The investigation was started with material from plants grown during the spring of 1926 and the observations checked from material obtained during the spring of 1927. For the host plant I used the tomato *Lycopersicum esculentum*. The bacteria[3] used were grown from two to four days on nutrient agar slants in tubes and inoculated into the internodes of the young stems of the tomato. The tomato plants were grown in the greenhouse and hot bed under optimum conditions and were in all cases vigorous, healthy young plants. Numerous plants inoculated with these cultures as checks developed the typical tumors of the crown gall disease. Inoculations were made near the tips of the stems in internodes about 1 cm. in length. In making the inoculations, no attempt was made to limit the dosage of bacteria. In fact, maximum dosage was usual. Later work has convinced me that the first internode above the cotyledons of young seedlings about 3 inches in height furnishes the most uniform material. The material for microscopic study was taken at intervals of 15, 30, and 45 minutes, 1, 1½, 2, 3, 4, 5, 6, 9, 12, 18, 24, and 48 hours after inoculating. Pieces of the stem about 1 cm. in length, each containing the inoculation puncture, were killed in Flemmings' solution and imbedded in paraffin. Especial care was taken to secure abundant material of the stages shortly after inoculation, especially within the first hour. Microtome sections of this material 7 to 15 microns thick were

[2] Acknowledgements are due Miss Frances P. Gibbons, Mrs. Helen D. Hill, and Miss Ruth I. Focht for assistance in parts of the microtechnical work. I am also indebted to my colleagues in the Department of Botany, The Pennsylvania State College and to Dr. C. R. Orton of the Bayer Chemical Company, New York, for their criticism of this manuscript.

[3] The culture of *B. tumefaciens* used in this investigation was one isolated from *Geranium soleroi* and furnished to me by Dr. H. W. Thurston, Jr. Cultures of the causal organism used in the checks were sent to me by Miss Nellie A. Brown, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C. Throughout this paper I have used the name, *Bacterium tumefaciens*, according to the nomenclature proposed by Dr. Erwin F. Smith. I recognize that the older name *Pseudomonas tumefaciens* is extensively used and also that use of the newer name, *Phytomonas tumefaciens*, may in time supersede the others.

stained in Flemmings' triple stain. This staining method is an adaptation of Nixon's (3) technique for staining bacteria *in situ*.

During 1927, I checked the entire investigation from material secured during the spring of that year. In the checks I used the castor bean, *Ricinus communis*, and the flowering tobacco, *Nicotiana affinis* (Sanderii hybrids), as additional hosts and a different strain of bacteria known as "peach" secured from the laboratory of Dr. Erwin F. Smith. I also used different microtechnical methods supplementing the Flemmings' solution with the alcohol-acetic acid-formalin and Petrunkewitsch fixing solutions and staining with iron-alum haematoxylin in addition to the Flemmings' triple strain.

The Method and Path of Migration

The young tomato stem has the following tissues: a single-layered epidermis, a sub-epidermal, chlorophyllose parenchyma tissue from two to four cells in thickness, a collenchyma four or five cells in thickness, an inner cortex, a narrow vascular region consisting of bi-collateral bundles with scattered anastomosing phloem strands, and finally a relatively extensive central pith made up of large parenchyma cells with large elongated intercellular spaces which are of triangular shape in the transverse section. When considering the migration of *B. tumefaciens* in the stem of the tomato, three regions may be considered: the pith, the subepidermal tissues, and the xylem, if the latter is injured.

When the inoculating needle bearing a quantity of *B. tumefaciens* penetrates the young stem, a considerable number of cells of the various tissues of the host are destroyed. Microscopic examination of sections of the stem through the punctured region reveals every gradation of injury to the cells on the margin of the wound from lack of injury to total destruction. The bacteria are to be found growing in the mass of injured tissue. Most of the sections studied showed that the bacteria were growing in the tissues and not merely occupying the cavity formed by the needle. Very rarely the cavity resulting from the puncture was found to be filled with bacteria. Cells on the margin of the wound which were slightly ruptured but not completely disorganized by the passage of the needle were almost invariably found filled with bacteria within one hour after inoculation.

I found that the bacteria migrate as zoogloeae penetrating the pith and the sub-epidermal tissue (Plate XII, A, B, and C). The zoogloeae form in the mass of cells which were injured by the puncture of the needle. They then move vertically through the intercellular spaces. I was able to trace them through the pith both upward and downward from the injured cells around the puncture. In places in pith tissue some slight radial and

tangential migration was found. In the intercellular spaces of the pith I found elongated zoogloeae perfectly continuous for distances from 0.35 mm. to 0.56 mm. and continuous but for slight interruptions for much greater distances. These zoogloeae were found in fairly large numbers. My observations lead me to conclude that the zoogloeae arise in the injured tissue in the region of the puncture and penetrate the intercellular spaces as isolated individual masses. The zoogloeae rarely occur as an anastomosing system in the tissues of the pith. Branched zoogloeae occur in the sub-epidermal region, where the anatomical structure is favorable for such development. The measurements given in a following section are based upon more than 80 distinct zoogloeae. In some instances a single zoogloea passes through an intercellular space in a given region of pith. In others a whole group of zoogloeae occurs in a restricted area. In such an area bacteria are in contact with some of the cells at every possible point since the zoogloeae may occupy almost all the intercellular spaces in a restricted area. The zoogloeae do not always completely occupy the intercellular space, but in some places they do completely occupy it and there they assume its shape.

Some of the physical properties of the zoogloeae may be inferred from a study of their appearance and behavior. The zoogloeae consist of a mass of bacteria imbedded in or surrounded by a jelly-like matrix. Apparently the form of these masses in tissue is dependent upon the size, shape, and general structure of the intercellular spaces in which they chance to be growing (Plate XII, A). Zoogloeae growing in the long narrow intercellular spaces of the pith of the tomato stem assume their shape and appear as long narrow strands (Plate XII, C). Zoogloeae growing in the sub-epidermal tissues assume a variety of forms. Most of the bacterial masses in the latter tissue are short and broad, and, where the shape of the intercellular spaces is favorable for such development, branching zoogloeae may be found.

In *B. tumefaciens* the matrix surrounding the densely crowded bacteria appears to be of a fluid nature. The margins and advancing tips of the zoogloeae are typically blunt and rounded in a convex manner with the outline sharp and clear (Plate XII, B and C). The form of the tip of the zoogloeae resembles the meniscus of a heavy fluid such as mercury. The form of the tip of the zoogloeae in the narrow intercellular space indicates that the matrix is of some substance not adhering to the walls of these spaces (Plate XII, B and C). The consistent progress of the zoogloeal masses in the intercellular spaces at successive intervals indicates that the physical nature of the matrix allows a free flowing movement. In tomato tissue the matrix of the zoogloea of *B. tumefaciens* is not deeply stained

with the Flemmings' triple stain although the bacteria and the margins of the matrix are clearly differentiated.

Migration of the bacteria through the uninjured tissues of the stem is by way of the intercellular spaces of the pith and the sub-epidermal tissues. In no case was I able to recognize bacteria within any uninjured cell. In the young tomato stem the optimum path of migration of *B. tumefaciens* is through the large intercellular spaces of the pith. The bacteria penetrate to a greater distance from the puncture through the pith than through any other kind of uninjured tissue. Migration in the pith region is almost entirely vertical with very little migration in a radial or tangential direction. In the tomato stem the layer of chlorophyllose parenchyma from two to four layers of cells in thickness and occupying a position between the epidermis and the collenchyma, is also readily penetrated. This tissue is composed of loose parenchyma cells with many large irregularly shaped intercellular spaces. It resembles the spongy mesophyll of a leaf. The invasion of this sub-epidermal tissue occurs early, within the first two hours following inoculation, and results in large masses of bacteria widespread through the large intercellular spaces (Plate XII, A). My observations, however, led me to believe that the bacteria do not penetrate so far from the puncture in this region as in that of the pith. Migration is vertical, radial, and tangential in the sub-epidermal region.

The failure of the bacteria to migrate through the collenchyma tissue is probably due to the absence of suitable intercellular spaces. Migration through the cortex and through the vascular region was also found to be rare and possibly does not occur in early stages. The intercellular spaces of the cortex are much smaller than those of the pith region, and those of the vascular tissues are still smaller than those found in the cortex. Since the early migration of the bacteria is confined to the pith and the sub-epidermal region, where relatively large intercellular spaces are found, it seems possible that the size of the intercellular spaces may be the factor determining the optimum path of migration. Migration of the bacteria through the xylem vessels was found to be of rather common occurrence but seemed to be confined entirely to those vessels which had been injured. Apparently the xylem is penetrated by the bacteria only when injury to some of the elements of this tissue has made an opening for the parasites, for the bacteria were only found within vessels which had been broken by the insertion of the inoculating needle through a bundle. Under such conditions the bacteria were found in the vessels both above and below the puncture, indicating that the migration was independent of the flow of sap in the vessels. This path of migration is not so frequently used as that through the intercellular spaces of the pith and the sub-epidermal tissues.

The Rate of Migration

In order to obtain some information concerning the rate of migration of the bacteria, I measured the maximum distance to which the zoogloeae had penetrated the intercellular spaces of the pith during various regular periods of time following the inoculation. The distance measured extended from the margin of the puncture to the farthest portion of the zoogloeal strand to be observed in the longitudinal section. A total of 83 such measurements was made on material secured from 15 minutes to 3 hours after inoculation. The progress of the bacteria, therefore, was measured in the early stages only, and I have no data on the rate of migration in later stages. All zoogloeae measured were located in the intercellular spaces of the pith. The material from which measurements were made had been grown at summer temperature in greenhouse and hotbed.

The migration of the bacteria through the intercellular spaces as zoogloeae is almost unbelievably rapid, though not as rapid as the motion of individual bacteria as determined by Riker (4). Migration was found to be well under way within 15 to 30 minutes after the bacteria were introduced into the stem, and far advanced within an hour. Table 1 shows the results of the measurements.

TABLE 1.—*The penetration of intercellular spaces by zoogloeae of Bacterium tumefaciens at different times after inoculation.*

Time (hours)	No. measurements	Average distance (mm.) penetrated by the zoogloeae	Rate a minute in mm.
¼	15	0.6008	.040
½	23	1.02	.034
1	6	1.3368	.022
1½	19	1.8178	.020
2	6	1.839	.015
3	14	2.6625	.015

The greatest distance traversed was 5.25 mm. in 3 hours, which is at a rate of 0.029 mm. a minute. The most rapid rate, however, is in the very earliest stages. The average rate for the first 15 minutes of 0.04 mm. a minute is almost twice as fast as the maximum rate for 3 hours. A growth curve[4] with the average measurements plotted against the time helps in visualizing the rate of growth (Fig. 1).

[4] I am indebted to Howard L. Clark for the graph illustrating the rate of growth of the zoogloeae.

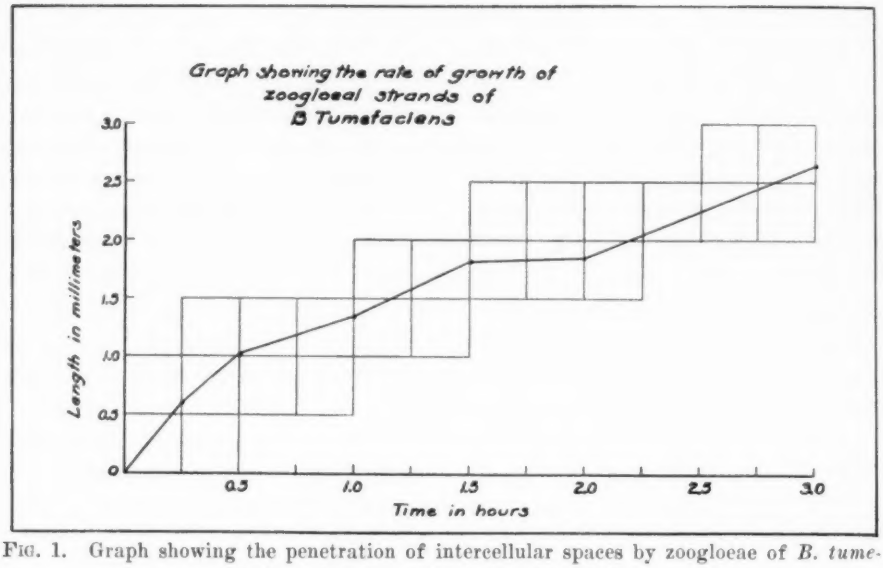

FIG. 1. Graph showing the penetration of intercellular spaces by zoogloeae of *B. tumefaciens* at various times after inoculation.

RESULTS OF CHECKS

As indicated in a former section, I carefully checked all phases of this investigation from material obtained in 1927. The results from the check material confirmed the results obtained in the tomato. I found zoogloeae in the intercellular spaces of both castor bean and tobacco tissue. The rates of migration of the zoogloeae were of the same magnitude as those measured in tomato.

DISCUSSION

The conception of the migration of the bacteria through the intercellular spaces as zoogloeae differs radically from the earlier ideas of Smith (11) who regarded the pathogen as an intracellular parasite whose migration was accomplished by the division and proliferation of the parasitized cells. It has been clearly shown by Riker (4), Robinson and Walkden (7), and by my investigation that *B. tumefaciens* occurs in the intercellular spaces. It is evident from my observations of the early stages of the migration resulting from inoculation that, after a few hours following their introduction into the tissues, the bacteria can be recognized only as widely scattered clumps clinging in the intercellular spaces. This is due to the fact that the rate of growth is very rapid and that after a few hours the tips of the zoogloeae pass through the tissues leaving occasionally a few scattered clumps of bacteria. Under these conditions it is very difficult to determine

the method of migration. This accounts for the difficulties experienced by previous investigators in finding the migrating bacteria.

The rate of migration of *B. tumefaciens*, or actually the rate of growth of the zoogloeae of this species, is so rapid that very early stages can be observed only in material killed within one hour after the bacteria have been introduced into the tissues. In the light of information concernig this rate, it becomes evident that the difficulty of the earlier investigators in tracing the first stages of migration was due to the fact that the material studied was too frequently taken long after the bacteria had passed far out into the tissue. An appreciation of the rate of migration of these bacteria indicates a change in technique.

The question really at issue here, then, is as to the medium in or through which the bacteria migrate from the puncture into the uninjured tissue. My own observation of numerous zoogloeae passing along through the intercellular spaces confirms those of Robinson and Walkden (7) as to the manner in which *B. tumefaciens* migrates through the tissues. No other method of migration of these bacteria has been reported as actually observed by any investigator. The conception of the migration of the bacteria as zoogloeae differs fundamentally from the idea of their migration as apparently implied by Riker (4), and (5), who throughout his discussion refers to the intercellular spaces as filled with liquid which furnishes a medium for the passage of the bacteria through the tissues. A realization of the difference between these two conceptions of the methods of migration is of fundamental importance in gaining an adequate conception of the behavior of *B. tumefaciens* in plant tissue. The importance of the conception of the migration of the bacteria as zoogloeae as against the earlier theories cannot be overemphasized. It is related to these as fact to hypothesis.

SUMMARY

1. This investigation shows that *B. tumefaciens*, the pathogen of crown gall, migrates as zoogloeae through the intercellular spaces of the subepidermal layer and those of the pith in young tomato plants.

2. The rate of growth of the zoogloeae through the intercellular spaces is from 0.029 mm. to 0.04 mm. a minute, the more rapid rate occurring in the early stages of the migration following the introduction of the bacteria into the young tomato stems. The rate is most rapid for the first 30 minutes following inoculation. After 3 hours the rate is reduced one-half.

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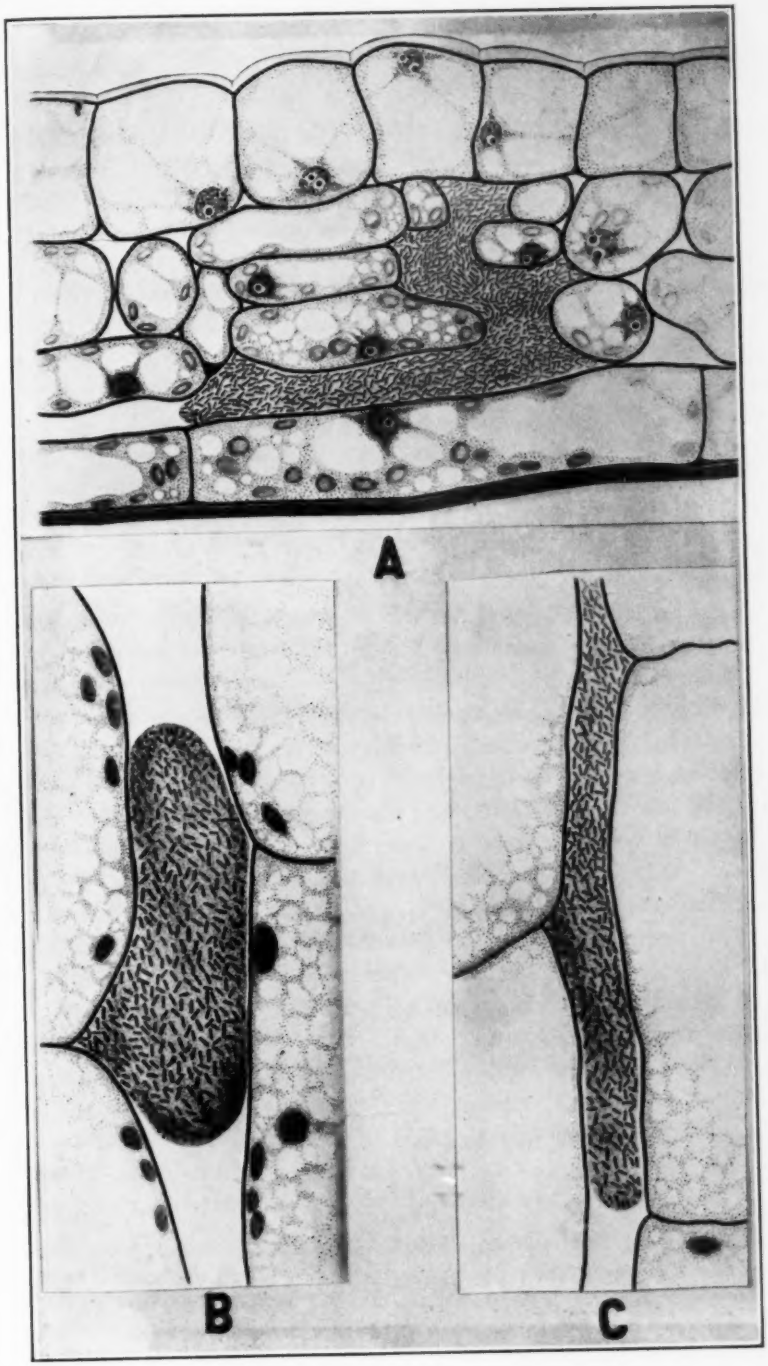
EXPLANATION OF PLATE XII

A. A radial longitudinal section through the outer portion of a tomato stem showing the relation of the zoogloeae to the intercellular spaces of the sub-epidermal tissue. The sub-epidermal tissue in this region consists of three or four layers of chlorophyllose cells situated between the epidermis (shown above) and the heavy walled collenchyma (below). The zoogloea occupies and conforms to the characteristically irregular intercellular space. The rounded tip of the zoogloea seen at the left indicates the vertical direction of its movement. Nuclei, cytoplasm, and chloroplasts are illustrated in the cells. Magnification about 1250.

B. A tangential longitudinal section through the sub-epidermal chlorophyllose parenchyma of the young tomato stem. The zoogloea has reached its present location by moving in a radial direction from an adjoining intercellular space. The radial direction is perpendicular to the plane of the paper. The two rounded tips indicate that this zoogloea is advancing vertically in both directions. Cytoplasm and chloroplasts are shown in the cells adjacent to the intercellular space containing the zoogloea. Magnification about 1250.

C. A longitudinal section through the central portion of the pith of a young tomato stem. An elongated zoogloea with the characteristically rounded tip is shown in the figure. As the zoogloea advances it assumes the shape of the narrow intercellular space which it occupies. Magnification about 1250.



UNIV OF MICH.

SOME FUNGI OF THE STEMPHYLIUM TYPE AND THEIR RELATION TO APPLE ROTS[1]

GEORGE A. NEWTON

In the Northwest the storage decays of apples are roughly classified by the market into the following five groups: blue mold, gray mold, anthracnose, false anthracnose, and Alternaria rot. It has long been known that the fungi causing the dark brown, or black, decayed areas roughly classified as Alternaria rot do not all belong to the genus *Alternaria*. To belong to that genus, according to Elliott (1), fungi must have the following characteristic essentials: "Conidiophores solitary or fasciculate, erect or subdecumbent, simple or branched, generally short, colored, conidia muriform, often with few longitudinal septa, ovate, obclavate or elongate, always with more or less definitely pointed apex, often long-beaked, colored, under favorable conditions forming chains." Spores from Alternaria-like cultures which are globular, sarcinaeform curved, or oblong, without apex or beak, he places in the genus *Stemphylium*.

With a view to studying in greater detail the different fungi causing apple rots in general, and the so-called Alternaria rot in particular, the fungi from decayed areas of several hundred apples from different localities of the state were isolated at the Washington Agricultural Experiment Station during 1925–26. The dark decayed areas referred to by the market in the Northwest as Alternaria rots yielded *Cladosporium*, *Phomopsis*, and *Stemphylium* besides *Alternaria*. The various isolations of *Stemphylium* were grouped in two types believed to represent species.

Two typical strains of rot-producing organisms were selected for special study. One of them (culture 4714–8B) produced abundant, colored, muriform conidia clustered on geniculate conidiophores, but never any perithecia; the other (culture 4716–6) produced conidia more sparingly. The conidia of the latter were colored, muriform, and usually borne singly on conidiophores that were slightly inflated at point of attachment to the conidium. In culture it usually produced from few to many perithecia, whose asci each contained eight muriform, yellow ascospores of the Pleospora type.

[1] Abridgement of a thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Agriculture at the State College of Washington.

The writer wishes to express his indebtedness to Dr. F. D. Heald, under whose direction this study was made, and to Professor B. F. Dana, for their helpful suggestions and inspiration throughout the work; also to Dr. Hannah C. Aase for her aid in the histological studies.

HISTORICAL REVIEW

In 1920 Horne (2) conducted an extensive investigation to determine the cause of spotting of apples in Great Britain. Among other fungi he found a species of *Pleospora* to which he gave the name *Pleospora pomorum*. He described the conidial form of this fungus as a *Stemphylium*.

Recently Rose and Butler (4) have isolated a species of *Pleospora* from decayed lemons from California, and from apples from Washington. They believe the fungus to be *Pleospora herbarum*, variety *citrorum*, and the conidial form they classified as a *Macrosporium*. They proved the pathogenicity of the fungus by cross inoculation.

RESULTS OF EXPERIMENTS

In the course of the experiments at Washington Experiment Station it was found that the *Pleospora* tended to lose its power to produce perithecia when carried long on rich media if frequently renewed. However, cultures made from a poured plate of ascospores that had not been renewed for several months would again produce perithecia (Fig. 4).

TABLE 1.—*The growth of cultures from single-spore isolations of Pleospora mali and Stemphylium congestum after seven days on various agars and at various temperatures*

Inoculum	Medium	Growth in centimeters at temperature of			
		30° C.	26° C.	20° C.	14° C.
Pleospora mali	2% dextrose potato agar	5.0	4.5	3.5	2.0
	2% dextrose beef agar	5.0	5.0	4.0	2.0
	Apple agar	4.0	6.0	4.0	2.0
	2% dextrose apple agar	6.5	6.5	4.0	2.0
	Prune agar	5.5	5.5	3.5	1.5
	Cornmeal agar	5.5	4.5	3.0	1.5
	Oatmeal agar	5.5	4.5	3.5	1.0
Stemphylium congestum	2% dextrose potato agar	5.5	3.5	2.5	1.5
	2% dextrose beef agar	4.5	3.5	3.0	1.5
	Apple agar	4.5	4.5	3.0	1.5
	2% dextrose apple agar	5.0	5.0	4.0	1.75
	Prune agar	3.5	4.0	3.0	1.5
	Cornmeal agar	3.0	3.0	3.0	1.5
	Oatmeal agar	6.5	6.0	4.0	1.5

Single spore isolations were made from both strains by means of the micro-loop. A small tube of glass was heated and so stretched and twisted as to form a microscopic loop. A suspension was made of spores to be isolated, and a small drop of the suspension placed on a sterile slide under the microscope. By use of the microscopic loop, single spores were picked out and planted in prepared plates of sterile potato dextrose agar.

Plantings taken from the single-spore isolation cultures and placed in various media at various temperatures showed some differences in amount of growth and in general appearance. (See tables 1 and 2 and figures 1 and 2.)

It is remarkable that at room temperature the cultures on prune and cornmeal agar produced perithecia within the week. All produced perithecia within 28 days, although all perithecia were not fertile at that

TABLE 2.—*The production of perithecia and the appearance of cultures from single-spore isolations of Pleospora mali and Stemphylium congestum after seven days on various agars at room temperature*

Inoculum	Medium	Perithecia	General appearance
Pleospora mali	2% dextrose potato agar	None	Gray, fluffy on top, medium density; dark underneath.
	2% dextrose beef agar	do	Almost white on top, felt-like; yellowish and black underneath.
	Apple agar	Few	Gray, fluffy on top, thin growth; gray underneath.
	2% dextrose apple agar	None	Gray, fluffy, whitish center on top; gray underneath.
	Prune agar	Present in all plates	Gray, thin growth on top; gray underneath.
	Cornmeal agar	do	Dark-gray, thin growth on top; dark-gray on bottom.
	Oatmeal agar	None	Gray, whitish center, dense on top; dark-gray on bottom.
Stemphylium congestum	2% dextrose potato agar	None	Black, dense growth on top; black underneath.
	2% dextrose beef agar	do	Black, dense growth on top; black and yellowish underneath.
	Apple agar	do	Dark, medium density on top; dark underneath.
	2% dextrose apple agar	do	Dark, medium density on top; dark underneath.
	Prune agar	do	Dark, medium density on top; dark underneath.
	Cornmeal agar	do	Dark, thin growth on top; dark underneath.
	Oatmeal agar	do	Black, dense growth on top; black underneath.

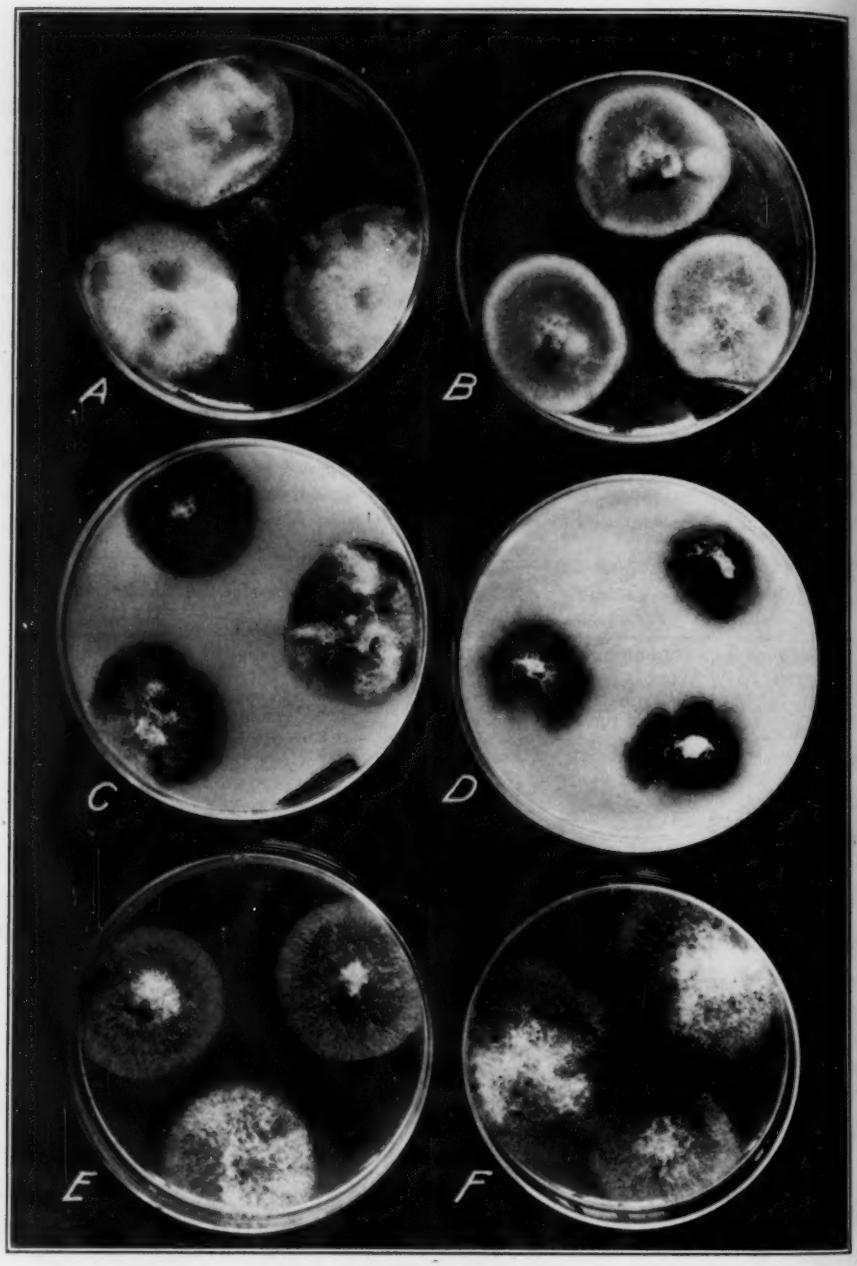


FIG. 1.—Plate cultures of *Pleospora* 4716–6 (*Pleospora mali*) from single conidiospore isolation culture. Grown eight days at temperature of 20° C. A, Apple agar; B, prune agar; C, cornmeal agar; D, oatmeal agar; E, potato dextrose agar; F, beef bouillon dextrose agar.

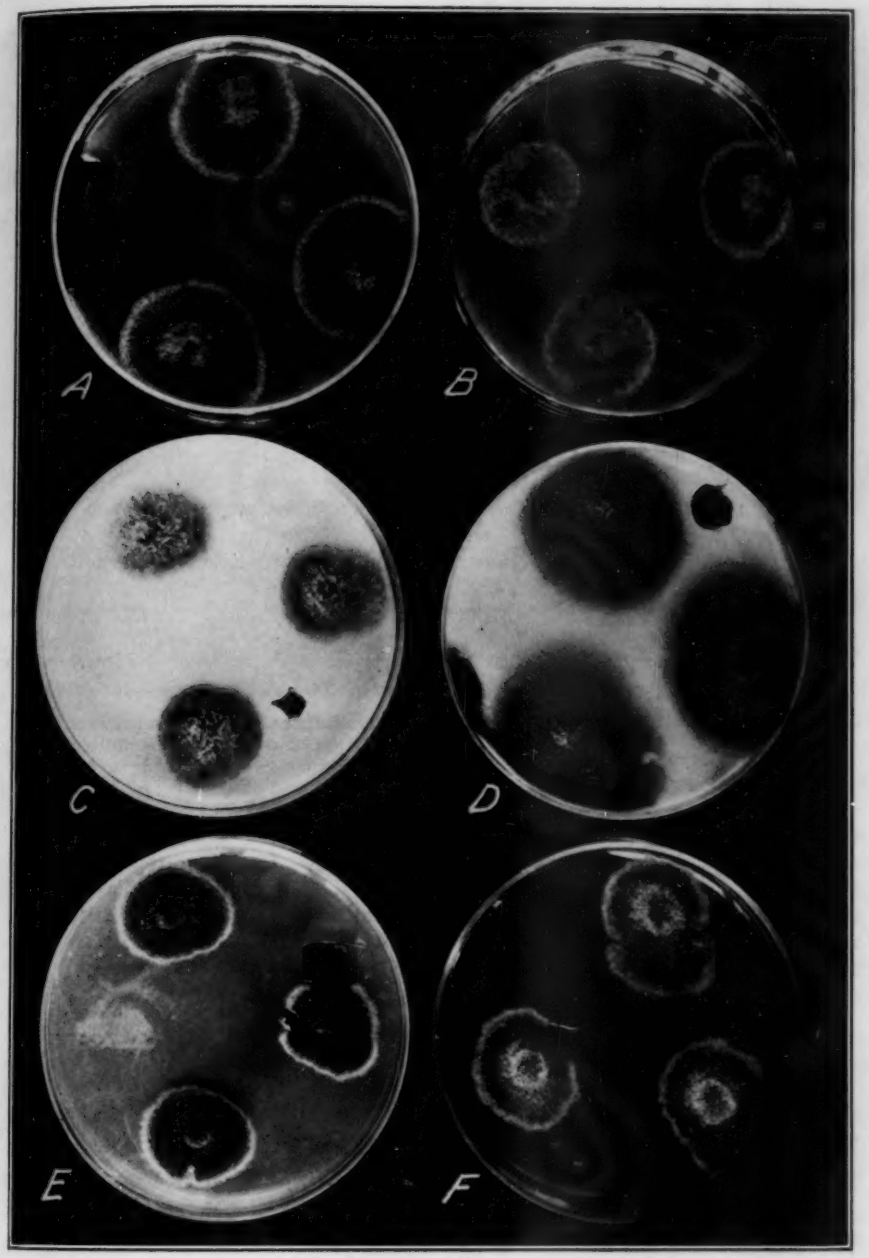


FIG. 2.—Plate cultures of 4714–8B (non-perithecium—producing *Stemphylium*) from single spore culture. Grown for the same length of time, and at the same temperature as the cultures in figure 1. A, Apple agar; B, prune agar; C, cornmeal agar; D, oatmeal agar; E, potato dextrose agar; F, beef bouillon dextrose agar.

time. At the end of 48 days nearly all cultures held at 15° C. and 21° C. had produced fertile perithecia. (See tables 3 and 4.)

TABLE 3.—*The production and condition of perithecia in cultures of Pleospora mali (culture 4716–6) grown on various media and at different temperatures for 30 days*

Medium	Quantity and condition of perithecia at temperatures of			
	15° C.	21° C.	27° C.	30° C.
Cornmeal agar	Many; sterile	Many; fertile	Many; sterile	Very few; sterile
Apple agar	Few; sterile	Few; sterile	Very few; sterile	Very few; sterile
Prune agar	Few; sterile	Few; sterile	Few; sterile	Few; sterile
2% dextrose potato agar	Very few; sterile	Few; sterile	Very few; sterile	None
2% dextrose apple agar	Very few; sterile	Few; sterile	Very few; sterile	Very few; sterile
2% dextrose beef agar	Very few; sterile	Small, very few; sterile	Small, very few; sterile	None
Oatmeal agar	Very few; sterile	Few; fertile	Few; sterile	None

TABLE 4.—*The production and condition of perithecia in cultures of Pleospora mali (culture 4716–6) grown on various media and at different temperatures for 48 days*

Medium	Quantity and condition of perithecia at temperature of	
	15° C.	21° C.
Cornmeal agar	Many; fertile	Many; fertile
Apple agar	Few; fertile	Few; sterile
Prune agar	Few; fertile	Few; sterile
2% dextrose potato agar	Very few; fertile	Very few; fertile
2% dextrose apple agar	Very few; sterile	Few; sterile
2% dextrose beef agar	Very few, small; sterile	Few; sterile
Oatmeal agar	Very few; fertile	Few; sterile

The results of inoculating Jonathan apples with the single-spore isolation cultures are given in table 5, and also shown in figure 3. Comparison is here made with *Alternaria* and *Penicillium* as agents of apple decay. The *Penicillium* causes the most rapid decay at the lower temperatures and at room temperature, but at 30° C. *Alternaria* causes the most rapid decay. There is not much difference in the rate of decay caused by the *Pleospora* and the *Stemphylium*. The results of keeping inoculated Rome apples at temperatures ranging around the freezing point are given in table 6.

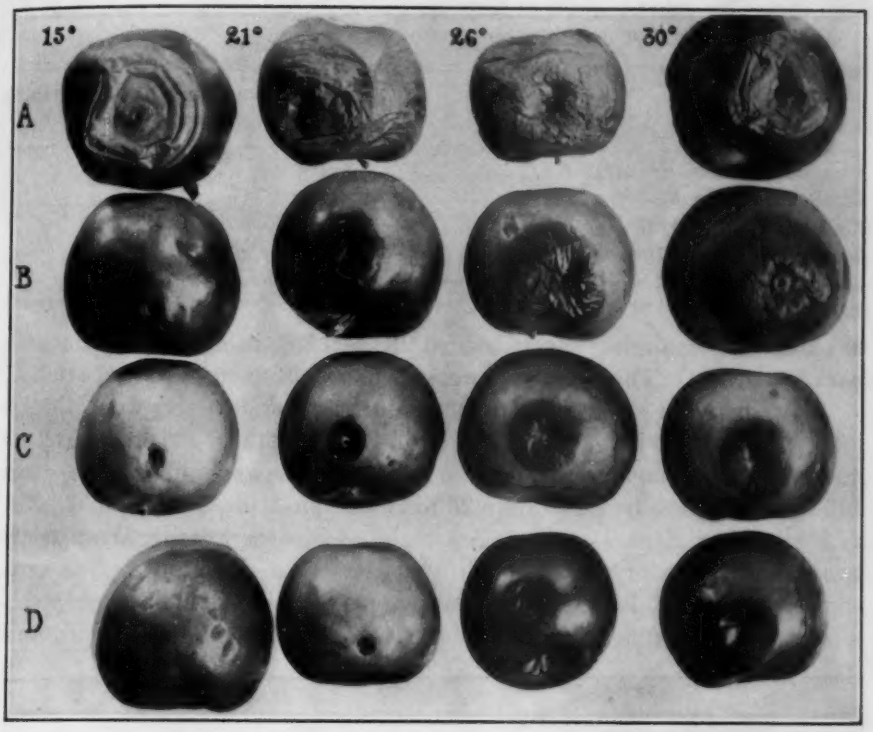


FIG. 3.—Jonathan apples inoculated March 25, 1927, with (A) *Penicillium*, (B) *Alternaria*, (C) *Pleospora* (4716–6), and (D) *Stemphylium* (4714–8B); and kept for two weeks at 15°, 21°, 26°, and 30° C.

MORPHOLOGY OF PLEOSPORA MALI

Some perithecia containing asci, and growing on an artificial medium, were killed in a solution of acetic acid in alcohol and dehydrated with

TABLE 5.—*The extent of decay in Jonathan apples two weeks after inoculation with single-spore isolation cultures of various rot-producing organisms*

Inoculum	Diameter in centimeters of decayed area at temperatures of			
	15° C.	21° C.	26° C.	30° C.
Penicillium	5.0	6.0	Whole apple	5.0
Alternaria	2.0	2.75	4.5	6.0
Stemphylium congestum (4714–8B)	0.5	1.75	3.5	2.5
Pleospora mali culture 4716–6	0.75	1.5	3.5	3.0
Control	0.0	0.0	0.0	0.0

TABLE 6.—*The extent of decay in Rome apples kept in cold storage (−2° C. to 2° C.) for 75 days after inoculation with Pleospora or Stemphylium*

Inoculum	Diameter of decayed area in centimeters
Pleospora mali culture 4716–6	2.0
Pleospora culture 4716–7	2.0
Pleospora culture 4727–1	1.0
Stemphylium congestum (4714–8B)	2.0
Stemphylium culture 4734–E	2.5

alcohol. The material was imbedded in paraffin, using chloroform as a paraffin solvent. The perithecia were then sectioned, stained, and studied.

The perithecia are sub-emergent bodies more or less spherical, though often pear-shaped (Fig. 6). Each perithecium contains numerous asci, as many as 40 asci having been counted in some. The asci range in size from 146 to 200 microns long and from 26 to 29 microns wide, averaging 173.0 by 27.5 microns. Each ascus contains eight ascospores usually arranged in two rather irregular rows, though sometimes in a single row. The ascospores are yellow, later turning darker, muriform, usually six or seven-septate, often constricted in the middle. They have one to two longitudinal

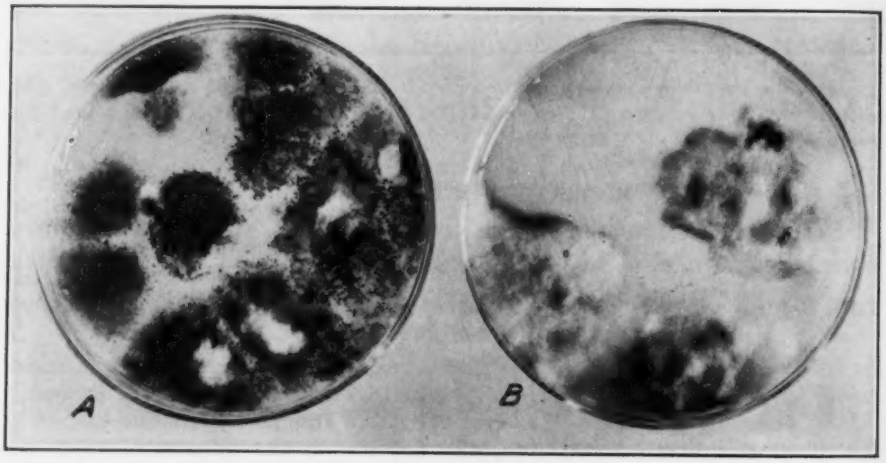


FIG. 4.—Plate cultures of 4716–6 and 4753–E2B, grown on prune agar at room temperature. Both are the perithecium-producing or Pleospora type. A, from a single spore isolation from conidia produced on a poured plate of ascospores from old dry plate culture that had not been renewed for several months. B, from stock culture carried about two years in ice box on potato dextrose agar changed about once a month. Though both were originally perithecium-producing, only the one from the single spore isolation from the cold culture now produces perithecia.

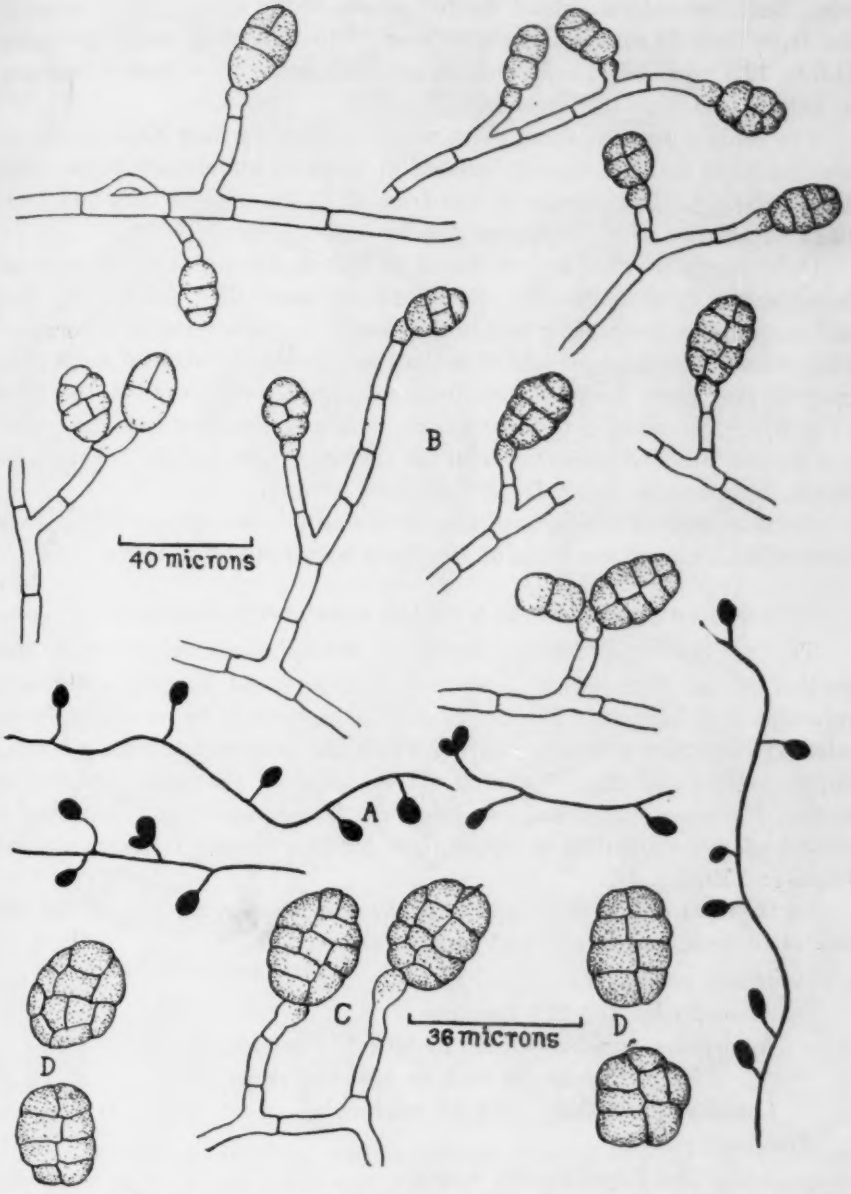


FIG. 5.—*Pleospora mali.* A, diagram to show arrangement of conidia and conidiophores; B, camera lucida drawing of formation of conidia on potato dextrose agar in Van Tieghem cell; C, camera lucida drawing of formation of conidia in plate culture of potato dextrose agar; D, camera lucida drawing of separate conidia.

septa, but these seldom extend the full length of the spore. They range in size from 28 to 34 microns long and from 11 to 14 microns wide, averaging 31.0 by 12.5 microns. The hyphae in artificial media often have a tendency to gather into rope-like strands.

The conidia are muriform, often warty, yellow, turning black, borne on conidiophores that are usually inflated at point of attachment to the conidium (Fig. 5). They range in size from 21 to 36 microns long and from 12 to 24 microns wide, averaging 28.5 by 18.0 microns.

Only sterile perithecia were found in lesions on apples which were infected naturally or artificially. Some of these were killed, fixed, embedded, and sectioned as described above in the case of the perithecia from cultures. They were of the same general shape as those grown in artificial media. In general they were located just underneath the epidermis of the fruit (Fig. 6). The presence of these minute bodies just underneath the epidermis gives a pimpled appearance to the surface of the cuticle. Some perithecia, however, are found deeply imbedded within the fruit.

Cross sections of the decayed area of the apple show the mycelium to be intercellular, though some bits of mycelium were found within the cells.

TAXONOMY OF PLEOSPORA MALI AND STEMPHYLIUM CONGESTUM

The perithecium-producing forms of *Stemphylium* which have been studied at the Washington Agricultural Experiment Station are clearly referable to *Pleospora*. The forms studied appear to be very closely related to *Pleospora pomorum* Horne, which has been isolated from spotted apples in England (2). The only other species of *Pleospora* reported on apples, *Pleospora herbarum*, var. *citrorum*, is too imperfectly described to permit of a certain differentiation, but for the present it seems distinct (Rose and Butler, 4).

Our species differs especially from *P. pomorum* in the size of the asci and ascospores, and in size and form of the conidia.

Pleospora pomorum

Asci—23 by 160–220 microns
Ascospores—golden—31–40 by 10.3–15.5 microns
brown—28–34.5 by 13–14 microns
Conidia—23–38.5 by 13.5–23 microns

Pleospora mali

Asci—26–29 by 146–200 microns
Ascospores—28–34 by 11–14 microns
Conidia—21–36 by 12–24 microns

The conidia of our species, which are also of the *Stemphylium* type, are more elongated than the sphaero-quadrilateral type figured by Horne and Horne (3).

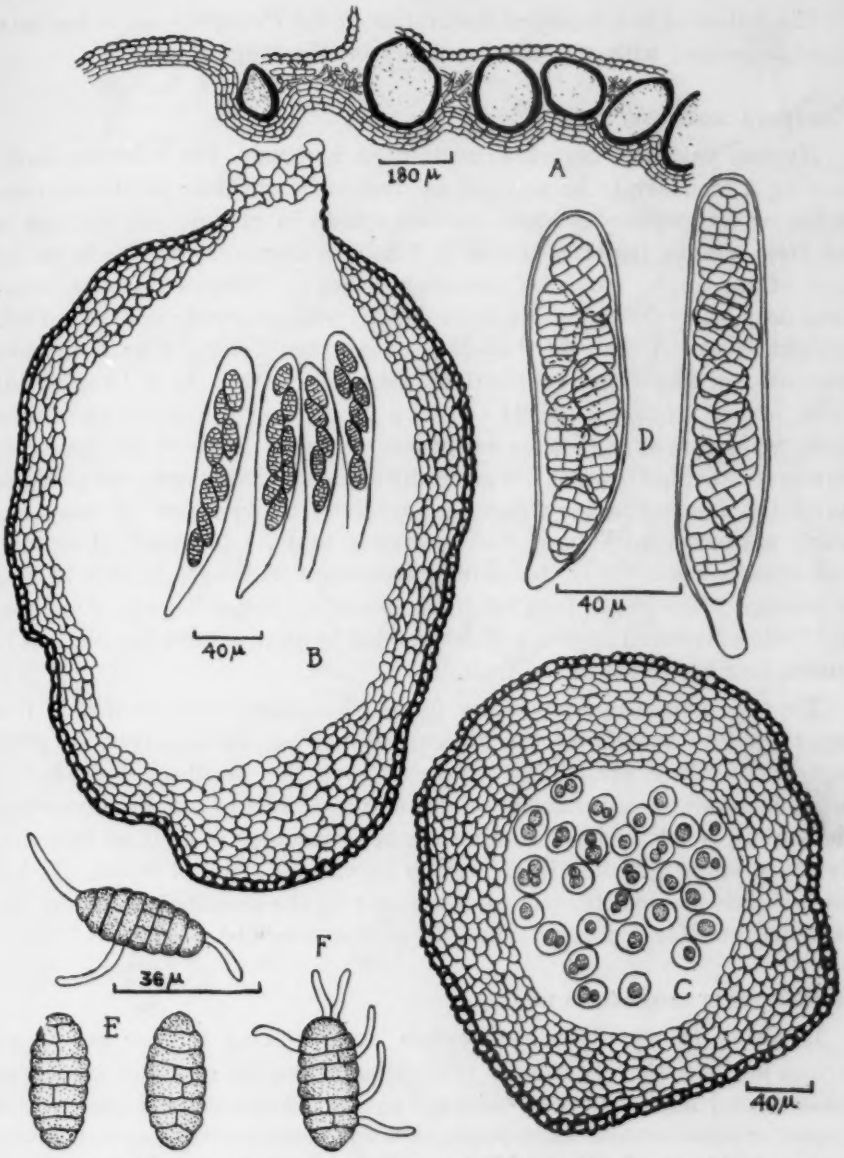


FIG. 6.—*Pleospora mali.* A, semi-diagrammatic drawing of perithecia underneath epidermis of apple; B, longitudinal section through perithecium grown in potato dextrose agar; C, cross section through perithecium grown in potato dextrose agar, showing cross sections of asci and ascospores; D, asci containing ascospores from perithecia grown on potato dextrose agar; E, free ascospores; F, germination of ascospores after 6 hours in Van Tieghem cell on potato dextrose agar.

The following is a technical description of the *Pleospora* which has been found associated with, and causing decay in, Washington apples.

Pleospora mali nov. sp.

Hyphae variously branched, septate, at first pale, but becoming dark, showing a tendency to be grouped in strands in cultures; perithecia none to few or very numerous in the various strains in culture, and varying in size from minute, imperfect forms to 1 mm. in diameter; perithecia on the fruit of the apple, immersed becoming erumpent, always infertile, sometimes deep below the epidermis, but generally subepidermal; asci cylindrical, straight, 8-spored, 146–200 × 26–29 μ or average 173 × 27.5 μ; ascospores ovate-oblong, muriform, subbiseriate, 7-septate, with 1 to 2 longitudinal walls, orange to brown, 28–34 × 11–14 μ or average 31.0 × 12.5 μ; conidia of Stemphylium type, few or numerous, terminal on short or elongated, septate conidiophores which are generally expanded below the spore; conidia muriform, fuscous to very dark, ovate-oblong to irregular or sometimes nearly sphaero-quadrilateral, 1–3 transverse septa, 1 longitudinal septum, wall smooth when young, becoming tuberculate with age, 21–36 × 12–24 μ or average 28.5 × 18 μ. Isolated from lesions on Rome Beauty, Jonathan, and Yellow Newtown apples, and determined by pure culture inoculations to cause a firm brown rot of the fruit.

The non-perithecium-producing forms which have been studied in this paper produce conidia of the *Macrosporium-Stemphylium* type in great abundance. They are clearly distinct from the perithecium-producing forms, as shown by cultural characters and the manner of conidial formation. The various isolations have shown a marked constancy in cultural behavior, always producing conidia in abundance on various types of media. It has not been possible to refer our species to any of the described species of the Stemphylium-Macrosporium type. It will be described as new.

Stemphylium congestum nov. sp.

Hyphae variously branched, septate, dark, making a dense growth on various media with very copious production of conidia; conidia muriform, sphaero-quadrilateral to ovate-oblong, 1 to 3 transverse septa, 1 longitudinal septum or none, smooth when young, but tuberculate with age, and becoming nearly black, 17–30 × 12–19 μ, average 23.5 × 15.5 μ; conidia produced acrogenously on simple or slightly branched septate conidiophores, never single but accumulating in botryose clusters of two to many. Isolated from lesions on Rome Beauty and Jonathan apples and determined by pure culture inoculations to cause a firm brown rot of the fruit.

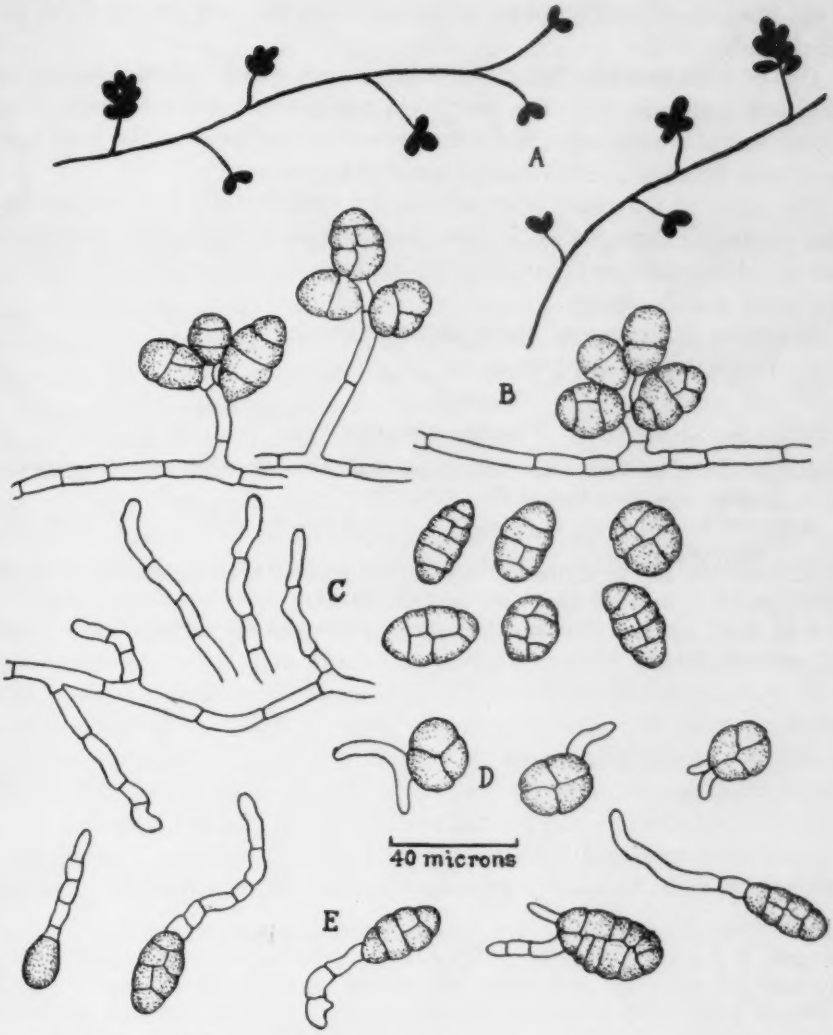


FIG. 7.—*Stemphylium congestum.* A, diagram showing arrangement of conidia and conidiophores; B, formation of conidia on potato dextrose agar in Van Tieghem cell; C, drawing showing conidia and conidiophores grown on potato dextrose agar plate; D, germination of conidia after 6 hours in Van Tieghem cell on potato dextrose agar; E, germination of conidia after 3 hours in distilled water.

SUMMARY

The black rots generally referred to by market inspection reports in the Northwest as "Alternaria rot" have been shown to be caused by several different fungi, among which are two different species producing conidia

of the Macrosporium-Stemphylium type. These two were selected for detailed study.

One of these is a perithecium-producing form which is here described as *Pleospora mali* nov. sp. The perithecia produced in culture media form mature asci and ascospores, while the perithecia produced on the fruit have never been found to contain mature asci nor ascospores.

The other of the two species selected for special study is a non-perithecium-producing form which is here described as *Stemphylium congestum* nov. sp. Its conidia are usually borne in dense clusters on the side and top of crooked conidiophores.

WESTERN WASHINGTON EXPERIMENT STATION,
PUYALLUP, WASHINGTON.

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NEW PHYSIOLOGIC FORMS OF TILLETIA LEVIS AND T. TRITICI[1]

E. F. GAINES[2]

Stinking smut has long been one of the major diseases of wheat and second only to the rusts in its destructiveness. In the United States the amount of smutty wheat coming to market during recent years is cause for grave concern. In the Pacific Northwest as much as 50 per cent of the cars coming to the terminal shipping points are given a smut dockage.

When the Western agronomists assembled at Moro, Oregon, for their annual meeting, June 27, 1927, Superintendent D. E. Stephens pointed out that there was something new to be seen in the smut resistance plots. Selections of Turkey wheat that had been resistant in former years contained so much smut that one could scarcely find a good wheat head. Mr. Stephens had obtained the inoculum, presumably a mixture of *Tilletia tritici* and *T. levis*, with which the seed had been inoculated from the Grain Supervision Office of the Bureau of Agricultural Economics, at Portland, Ore. Even such wheats as Ridit, Albit, Martin, and White Odessa contained from 7.7 to 51.5 per cent of smutted heads (7). These wheats had been practically smut-free in similar tests for years. In contrast with this result at Moro, the same varieties inoculated with local *T. tritici* at Pullman, Washington, did not show a trace of smut. However, a 20-acre field of Albit wheat on the College Farm at Pullman did contain 0.2 per cent of smutted heads. Since the seed was smut-free the year previous and had been sown without treatment, and since Albit had not produced a trace of smut during the

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previous four years in the smut tests in which local *T. tritici* inoculum was used, it seems possible in the light of evidence hereafter given that smut which attacked Albit was blown in from the great wheat belt to the West and infested the soil. The smut which occurred in Albit proved to be three-fourths *T. levis* and one-fourth *T. tritici*.

The branch experiment stations at Lind and Waterville, Wash., west of Pullman, likewise reported alarming increases in the amount of smut appearing in hitherto resistant varieties. At Lind, a highly resistant strain of Turkey (Wash. No. 326) that had been sown on the station farm without seed treatment for 10 years with no appreciable smut infection produced 75 per cent of smutted heads in 1927. The seed of this crop was inoculated with smut from a field of this same strain of Turkey which had been grown on the station in 1924. This is considered evidence that a new form of bunt to which this strain of Turkey is susceptible has been present at least since 1924. A microscopic examination showed it to be *T. levis*, and not *T. tritici*, which had been the prevailing smut on the Pacific Coast in former years.

A farmer near Waterville, Wash., who had been growing a resistant strain of Kharkof obtained from Sherman County, Ore., in 1918, had never considered seed treatment necessary for this particular variety. In 1925 a few heads of smut appeared in his crop. In 1926 the smut was much worse. The farmer, supposing the increase due to the unusually favorable season for smut, again sowed his crop that fall without treating the seed. In 1927 his entire crop of Kharkov averaged 60 per cent of smutted heads. Soil infestation with *T. tritici* is common in that locality, and a susceptible variety of wheat would have smutted badly every year. It seemed logical, therefore, to assume that a new form of smut to which Kharkof is susceptible came in, rather than to assume a change in the host. In support of this hypothesis a smut sample from this field was identified as *T. levis*.

Mr. B. B. Bayles (1), of the Judith Basin Substation, Moccasin, Montana, likewise reported heavy smut infection on heretofore resistant strains. At Moccasin there was 14.2 per cent of smut on Ridit, and from 80 to 90 per cent on such varieties as Kanred, Minhardi, and Minturki. This is the highest percentage of smut yet reported on Ridit in America. The inoculum for this experiment was obtained from a field of Turkey near the station and has been identified as *T. levis*.

In extensive smut surveys from 1918 to 1921, the bunt occurring in the Pacific Northwest was almost without exception identified as *T. tritici*, and wheat breeding experiments to develop resistant varieties have been conducted using spores of this species of the smut organism as inoculum. The above observations show that the other species, *T. levis*, is now present, that this latter is more virulent than the common forms before present, and that it has been increasing and spreading during recent years. Whether this is a

new physiologic form of *T. levis* different from that occurring east of the Rocky Mountains remains to be determined. Indirect evidence indicates that it is, for tests at Kansas from 1921 to 1923 showed Kanred to be very resistant to the common *T. levis* of that state, while the test at Moccasin in 1927 showed Kanred to be very susceptible.

It is known that the resistant wheats of America sometimes produce considerable smut in tests conducted in Europe, although in most cases they maintain their relative resistance. Rodenhiser and Stakman (4) obtained collections of both *T. tritici* and *T. levis* from foreign countries in 1925 and compared their virulence on Kota, Marquis, and Einkorn. A collection of *T. tritici* from Norway was much more virulent than one from New Zealand. A collection of *T. levis* from Hungary was more virulent on Einkorn than a collection from Egypt, but less virulent on Marquis. Both were distinct from the local *T. levis* of Minnesota.

The Welch Plant Breeding Experiment Station at Aberystwyth, Wales, tested six American wheats from the Washington State Experiment Station in comparison with four common English varieties, with the results shown in table 1.

TABLE 1.—*Comparative resistance to stinking smut of 6 American and 4 English wheats tested at Aberystwyth, Wales. Data furnished by Kathleen Sampson*

Variety	Percentage of infected plants, 1926	Percentage of smutted heads	
		1925	1926
American wheats			
Florence	38	7	20
White Odessa	9	—	4
Ridit	2	3	1
Turkey	12	17	11
Hussar	0	0	0
Martin	0	0	0
English wheats			
Hen Gymro	83	67	81
Yoeman	63	—	54
Standard Red	80	—	78
Little Joss	56	—	47

The American varieties were much more resistant than the English, although White Odessa and Ridit, which so far have been smut-free in tests at Pullman, did produce a small percentage of smutted heads. Martin and Hussar were smut-free, as they have been at Pullman. Florence and Turkey contained slightly more smut than is normal in similar tests at Pullman, but still would be classed as resistant wheats in comparison with the English varieties.

TABLE 2.—*Comparative resistance to Tilletia tritici of 11 American wheats at Cambridge, England, 1924–1927. Data from W. A. R. Dillon-Weston, University of Cambridge*

Variety and source	Year tested	Percentage of bunted heads
Sherman C. I. 4430 from Moro, Ore.	1924	1
	1925	1
	1926	2
	1927	8
Turkey C. I. 1558 from Manhattan, Kan.	1924	7
	1925	53
	1926	51
	1927	74
Kanred C. I. 5146 from Moccasin, Mont.	1924	10
	1925	27
	1926	18
Clackamas C. I. 6241 from Davis, Calif.	1925	8
	1926	15
	1927	56
Masters Perfection C. I. 4726 from Chico, Calif.	1924	6
	1925	4
	1926	23
	1927	59
White Odessa C. I. 4655 from Moro, Ore.	1924	2
	1925	0.3
	1926	0
	1927	9
Ridit C. I. 6703 from Moro, Ore.	1924	trace
	1925	0.7
	1926	1
	1927	4
Martin C. I. 4463 from Moro, Ore.	1924	0
	1925	0
	1926	0
	1927	0.5
Hussar C. I. 4643 from Moro, Ore.	1924	0
	1925	0
	1926	0.2
	1927	0.6
Fulcaster C. I. 6162	1925	26
	1926	89
Berkeley Rock	1925	0.5
	1926	1
	1927	2

At Cambridge, England, 11 varieties of American wheat were tested during the four years 1924 to 1927. The results are shown in table 2.

Sherman, White Odessa, Ridit, Martin, Hussar, and Berkeley Rock, which seldom showed a trace of smut in American tests, produced from 0.5 to 9 per cent of smutted heads. They were much more resistant, however, than the others, especially in 1926 and 1927. The increase in 1927 suggests either a very favorable season for smut or the introduction of a more virulent form. A test with *T. levis* in 1924 on four of the varieties, Sherman, Ridit, Martin, and Hussar, indicated little, if any, difference in its pathogenicity from that of *T. tritici*.

In 1925, Dr. Theodore Roemer visited the United States and arranged a cooperative experiment in which smut and wheat were exchanged between the University of Halle-Saale, Germany, and the State College of Washington at Pullman. The results at Pullman (2) in 1926 are shown in table 3.

TABLE 3.—*A comparison of German and American smut (T. tritici) in respect to infecting power on eight local Pacific Northwest wheats and four wheats from Halle-Saale, Germany, at Pullman, Washington, in 1926*

Variety	Source of Seed	Source of inoculum and percentage of bunted heads	
		America	Germany
Hybrid 128	Pullman, Washington	92	98
Triplet	do	79	84
Little Club	do	91	100
Turkey (Wash. No. 326)	do	2	36
Martin	do	trace	38
Ridit	do	0	1
Hussar	do	0	19
White Odessa	do	0	71
Hohenheimer (Behaart)	Halle-Saale, Germany	36	1
Hohenheimer 77 (Unbehaart)	do	33	0
Fürst Hatzfeldt	do	54	15
Heils Dickkopf	do	42	0

The preliminary experiments at Pullman in 1926 left no doubt as to the different physiologic specialization of the two collections of *T. tritici*. The German smut in every case was more virulent on the American wheats but less so on the German wheats. Of the 12 wheats tested, Ridit was the only one that was very resistant to both forms. White Odessa and Heils Dickkopf were the best differential testers. Each was immune from its native smut but produced 71 and 42 per cent, respectively, of smutted heads from the foreign inoculum.

Dr. Roemer enlarged his experiment at the University of Halle in 1927 to include smut collected from the various sections of Germany where wheat is grown, as well as samples from Hungary, Switzerland, Holland, Sweden, Denmark, and the United States. He used as hosts three resistant and two susceptible German wheats and five resistant American wheats; also three hybrid populations of a resistant-susceptible cross. Each test was replicated four times. There were 100 seeds sown in each row, making 400 seeds for each test, from which an average of 360 plants was obtained. The entire experiment of 13 varieties treated with smut from 15 sources represented 780 rows with a total of 70,200 plants. With Dr. Roemer's permission the data are given in table 4 in terms of smutted plants, including the plants containing both wheat and smut as well as those entirely smutted.

The inoculum from each locality was good, viable material, as is shown by the uniformly high infection on the susceptible varieties, Stocken and Panzer. The smut from Switzerland was the least virulent form, while the one from Cosel was, as measured by the average infection of all varieties, the most virulent. The two varieties that showed considerable resistance to all forms were Hohenheimer 77 and Ridit. The smut from Cosel infected Hohenheimer 77 to the extent of 34 per cent of the plants, but none of the other smuts produced more than 9 per cent in this variety. The smut from Bonn infected Ridit more than any other collection—24 per cent. Heils Dickkopf, Tubeuf, and Hussar were also quite resistant, having failed to become infected by one or more of the collections, but producing as much as 50 to 74 per cent when inoculated with spores from some one of the other sources. Martin, White Odessa, and Kharkov were more or less susceptible to some of the collections but resistant to others. The mixed populations of the resistant susceptible cross were intermediate, as might be expected, being most susceptible to Cosel smut and most resistant to the Hungarian smut, like Heils Dickkopf, the resistant parent.

In general, the smuts from Cosel, Breslau, Bonn, Weihenstephan, and Hohenheim, Germany, were the most virulent, those from Hungary, Göttingen, Germany, were intermediate, and the others were relatively weakly infective. The smut from Hungary attacked Martin and Kharkov more severely than any of the others, while the smut from Göttingen produced the highest infection of any on Panzer. Cosel smut was most severe for Heils Dickkopf and Hohenheimer 77. Tubeuf and White Odessa wheats were most severely infected with Breslau smut. Ridit was least resistant to Bonn smut, and Hussar least resistant to the smut from Hohenheim.

It would be premature to say on the basis of this experiment just how many physiologic forms are represented. The possibility of mixtures in both host and parasite is always a source of apprehension. There are five collections of stinking smut from which one or more of five different wheat

TABLE 4.—*The percentages of smutted plants obtained in 10 varieties and 3 hybrid populations of wheat inoculated with spores of 14 different collections of Tilletia tritici and one collection (Arlington Farm) of T. levis. Experiment conducted at Halle-Saale, Germany, by Theodore Roemer, 1927*

Wheat varieties	Cosel, Germany	Breslau, Germany	Bonn, Germany	Weihenstephan, Germany	Hohenheim, Germany	Hungary	Göttingen, Germany	Halle, Germany	Wageningen, Holland	East Prussia, Germany	Landskrona, Sweden	Lyngby, Denmark	Zurich, Switzerland	Pullman, Wash. U. S. A.	Arlington Farm, U. S. D. A.	Average
Hohenheimer 77	34	9	8	3	6	0	3	3	2	2	2	1	2	4	3	5.5
Ridit	7	10	24	10	14	5	3	5	6	3	1	2	0	0	6	6.4
Heils Dickkopf	50	15	46	29	16	0	8	8	9	9	8	3	10	6	12	15.3
Tubeuf	58	59	27	44	27	9	32	10	0	22	29	16	5	0	0	22.5
Hussar	50	46	26	32	74	57	25	1	0	8	1	19	0	1	6	23.1
Martin	77	61	64	70	72	78	27	1	1	26	3	20	12	1	10	34.9
Kharkov	52	62	38	60	38	69	51	40	52	27	34	32	44	21	37	43.8
White Odessa	83	85	84	67	80	79	74	32	7	33	2	16	40	8	38	48.5
Panzer	89	88	76	85	82	[a]	91	83	83	84	87	79	[a]	70	67	81.8[a]
Stocken	93	89	88	82	86	89	92	93	93	85	88	88	66	89	87	87.2
Helis Dickkopf × Hatzfeld a	62	40	35	34	32	8	29	35	40	17	17	18	28	17	18	28.7
Helis Dickkopf × Hatzfeld b	65	38	43	42	41	13	32	29	42	25	18	18	19	36	22	32.2
Helis Dickkopf × Hatzfeld c	59	48	39	48	29	14	34	31	26	28	20	23	23	44	22	32.5
Average	59.9	50.0	46.0	46.6	45.9	35.1[a]	38.5	28.5	27.8	28.4	23.8	25.8	20.7[a]	22.8	25.2	

[a] Percentage of smut in Panzer not shown for the collections from Hungary and Switzerland.

varieties are smut-free. Various hybrid combinations of the most resistant wheat varieties should give segregates more resistant than Ridit or Hohenheimer 77. Dr. Roemer, in his article (5), does not attempt to estimate the number of physiologic forms in his collections, but suggests that each coltion is probably a mixture of several pure lines. He emphasizes the need of using the most virulent collections for testing hybrid populations in order to isolate the most generally resistant segregates.

There is considerable evidence to show that what may be more virulent physiologic forms have been spreading rapidly in the United States during recent years. Since 1924, stinking smut has been the most destructive parasite of wheat in America. The enormous quantity of smutty wheat coming to market has induced the larger terminal elevators of the Central West, according to Shollenberger (6), to install washers for removing the smut. Smut has been on the increase, especially in Kansas, Colorado, and Nebraska, where hard red winter wheat is grown, as well as in Virginia and Pennsylvania, where soft red winter wheat predominates. The losses in these five states alone in 1926, according to Kirby (3), was 10 per cent in Kansas, 8 per cent in Colorado, 6 per cent in Nebraska, 6 per cent in Pennsylvania, and 5 per cent in Virginia. Taking the total wheat produced in these five states according to the 1926 United States Department of Agriculture Yearbook and multiplying by the percentages, the loss amounts to 20,659,460 bushels.

Kirby's estimates are corroborated in a general way by the percentage of carloads of wheat graded as smutty at the principal shipping posts, as shown in table 5.

Table 5 was compiled by the Office of Grain Investigations of the Bureau of Agricultural Economics. There are fluctuations from year to year, but the general trend is in the direction of increasing amounts of smut. The total average receipts at Kansas City, Omaha, Baltimore, and Philadelphia were 12, 16, 23, 26, and 28 per cent of smutty wheat for the five years 1923 to 1927. This is a consistent increase in which the amount has more than doubled in five years. In the Pacific Coast States, where seed treatment has been emphasized and where resistant varieties like Ridit are being introduced, there is a slight decline in the amount of smut since 1924. At Minneapolis in 1926 there was a sudden increase of cars grading smutty. This was confined almost entirely to the hard red winter and to the durum classes of wheat. Durum has always been considered resistant to smut but in 1927 at Minneapolis there were 22 per cent of the cars of durum, and 24 per cent of the cars of hard red winter wheat that graded smutty. The hard red spring class remained practically smut-free as in former years.

At Bozeman, Mont., there has been a rather rapid increase of smutty wheat from 12 per cent of cars grading smutty in 1923 to 47 per cent grad-

TABLE 5.—*Percentage of cars of wheat received at different markets which graded smutty. The shipments from July 1 to June 30 of the following year were taken as the crop year. (Less than 1 per cent not marked). Compiled by E. G. Boerner and F. C. Meier*

	1923	1924	1925	1926	July-October 1927
Astoria, Ore.	52	69	38	55	47
Baltimore, Md.	0.8	2	9	8	16
Bozeman, Mont.	12	19	17	34	47
Denver Colo.	21	25	36	25	19
Des Moines, Ia.	—	8	7	15	20
Detroit, Mich.	—	—	11	8	11
Duluth, Minn.	—	5	17	15	14
Indianapolis, Ind.	3	6	2	6	22
Kansas City, Kans.	8	11	10	22	14
Kearney, Neb.	4	35	54	28	12
Lawrenceburg, Ind.	6	37	38	44	19
Lincoln, Neb.	—	12	26	20	9
Los Angeles, Cal.	20	12	15	18	16
Louisville, Ken.	5	9	2	4	11
Minneapolis, Minn.	2.4	0.7	—	16	12
Ogden, Utah	14	21	29	33	31
Oklahoma City, Okla.	1	1	1	5	1
Omaha, Neb.	14	26	40	40	25
Philadelphia, Pa.	3	2	10	8	31
Portland, Ore.	45	60	30	42	40
Sacramento, Calif.	6	9	—	12	24
Seattle, Wash.	35	41	24	28	32
Stockton, Calif.	26	11	6	19	6
Tacoma, Wash.	42	54	30	35	32
Toledo, O.	1	3	2	2	11

ing smutty from July to October, 1927. The experiments at Moccasin, Mont., showing the presence of a strain of smut which attacks the hard red winter wheats with great virulence suggest a plausible explanation for the increase.

No doubt climatic conditions and cultural practices are responsible for wide fluctuations from year to year, but when the trend over a period of years is in the direction of increasing amounts of smut, and when carefully conducted experiments show forms of smut attacking types of wheat that were resistant in former years, the conclusion seems justified that the new forms of smut are responsible for some of this increase.

Tisdale, Leighty, and Boerner (8) have shown that *T. levis* is the most prevalent species east and south of the Dakotas, but since the prevalence of smut is increasing in these regions also, there is no reason to suppose that *T. levis* is less subject to physiologic specialization than *T. tritici*. In Dr. Roemer's experiments all forms were *T. tritici* except the smut from Arling-

ton Experiment Farm, Rosslyn, Va., which was *T. levis*. The smuts which attacked the Turkey, Kharkof, and other varieties so severely at Moccasin, Mont., Lind and Waterville, Wash., were all *T. levis*. The smut collected on Albit at Pullman, Wash., contained smooth and rough spores in the ratio of 3:1. The smut common to Pullman and the one that has been used in the breeding work of the Washington Station is *T. tritici*. In respect to the reaction on the resistant Turkeys, which probably represent a major part of the hard red winter wheat as a class, five forms may be assumed from analyzing the tests of the past two years. They may be listed as follows:

Tilletia tritici	Pullman form	Infects Turkey 5 per cent
do	German form	Infects Turkey 35 per cent
do	North Dakota form (6)	Infects Durum but not Turkey (?)
Tilletia levis	Old form	Infects Turkey 10 per cent
do	Eastern Washington form	Infects Turkey 75 per cent

This is probably a much simpler classification than subsequent experiments will show actually exist. The percentages of infection are purely hypothetical, since data under comparable conditions are not available. Experiments are in progress at several of the western experiment stations designed to give this information in 1928.

The most hopeful point of attack to meet this new situation is a greatly increased program of breeding for resistance to the new forms. In the meantime, increased emphasis needs to be placed on seed treatment and cultural practices to control or lessen the damage caused by stinking smut, the most destructive cereal disease in the United States.

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UNITED STATES DEPARTMENT OF AGRICULTURE.

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INFLUENCE OF ENVIRONMENTAL FACTORS ON THE SEASONAL PREVALENCE OF CORN SMUT[1,2]

F. R. Immer and J. J. Christensen[3]

INTRODUCTION

The influence of climatic factors on the development of corn smut, *Ustilago zeae* (Beck.) Ung., and the relative susceptibility of corn plants at various stages of development is imperfectly known. In recent years, however, the relation of these factors to infection and prevalence of *U. zeae* has received considerable attention.

Selby and Hickman (11), in 1897, concluded that corn smut was more prevalent under dry weather conditions. More recently, Potter and Melchers (10) and Coffman et al (4) have confirmed these observations.

Arthur and Stuart (1) and Piemeisel (9) concluded that humid weather was most favorable for smut infection. Potter and Melchers (10), on the other hand, did not believe that lack of moisture could be considered a limiting factor for smut infection, provided conditions were favorable to the growth of the host. Tisdale and Johnston (12) concluded from their greenhouse experiments that humid conditions favored infection. They were also of the opinion that high temperatures were conducive to infection, and that low temperature was one of the chief factors limiting infection.

Corn seedlings seldom become infected under natural field conditions. A number of investigators, however, have reported seedlings to be very susceptible when artificially inoculated. Tisdale and Johnston (12), by means of a hypodermic needle, injected young seedlings with a conidial suspension of *U. zeae,* and concluded that seedlings seemed to be more susceptible than older plants. They stated that strains of corn reacted the same way in the field as they did in the greenhouse when artificially inoculated.

Immer (6) readily infected corn seedlings by hypodermic inoculation in the greenhouse but found no appreciable correlation between seedling

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[2] This study was made as a part of the general program of breeding for disease resistance being conducted cooperatively by the sections of plant breeding and plant pathology.

[3] The authors wish to express their appreciation to Drs. H. K. Hayes and E. C. Stakman for help and criticism in preparing this paper.

susceptibility under greenhouse conditions and natural field infection. Griffiths (5) concluded that all strains of corn were susceptible to smut at any age if sporidial suspensions were injected into very young growing tissue.

Kühn (8), Brefeld (2), Arthur and Stuart (1), and Piemeisel (9) noted that corn seedlings once infected with *U. zeae* were usually killed by the organism. However, Brefeld, Piemeisel, and a number of other workers have concluded that seedlings are less susceptible than plants beyond the seedling stage.

It is obvious that there are rather conflicting reports on the effect of climatic factors upon infection and the prevalence of corn smut, and on the relative susceptibility of seedlings to infection. Therefore the investigations reported herein were undertaken.

METHODS AND MATERIAL

In studying the effect of certain environmental factors on the prevalence of smut, use was made of the percentages of smut-infected plants in selfed lines of corn grown in an artificially induced smut epidemic from 1922 to 1927, inclusive. The methods of planting the seed, producing the smut epidemic and taking notes have been described in detail in previous publications (6, 7). The reports of the United States Weather Bureau Office at St. Paul, Minnesota, were used as the source of information relative to the temperature, amount of sunshine in per cent of possible, and number of days on which precipitation occurred during the corn-growing period.

A study was made also of the correlations between the degree of smut infection of plants grown under smut epidemic conditions in the field with the extent of infection obtained by hypodermic inoculation of plants grown both in the field and greenhouse from seed of the same strains of corn. Seed of 34 lines from a recombination of 8 low smut lines was planted in the "smut plot" and the plants grown under artificially induced smut epidemic conditions. Twenty plants of each line were also grown in another field. When the plants were about 3 feet high, the growing point of each was inoculated hypodermically with a suspension of sporidia of 8 physiologic forms of *U. zeae*. Seedlings of these same 34 lines were grown also in sand in the greenhouse and inoculated hypodermically when about 6 inches high with the same 8 physiologic forms. The forms of smut used were M2 to M9, inclusive, all of which had been isolated from smut galls collected at University Farm.

In order to eliminate errors due to chance infection, notes were taken only on the number of galls formed at the point of inoculation for plants hypodermically inoculated in the field. The seed of each line grown in the

greenhouse was planted in three uniformly replicated rows on November 9, the seedlings were inoculated on November 30, and notes were taken on December 8. The seedlings were watered once with a nutrient solution. Only seedlings which developed smut galls were classed as infected. The temperature from date of inoculation until the notes were taken ranged from 75° to 80° F.

EXPERIMENTAL RESULTS

Graphs are presented in figure 1 for mean temperature, sunshine in per cent of possible, and number of days on which more than 0.01 inches of precipitation occurred during the months of July, August, and September. The data are summarized for each two weeks period. The graphs are constructed from averages for the first 15 days and the remainder of each

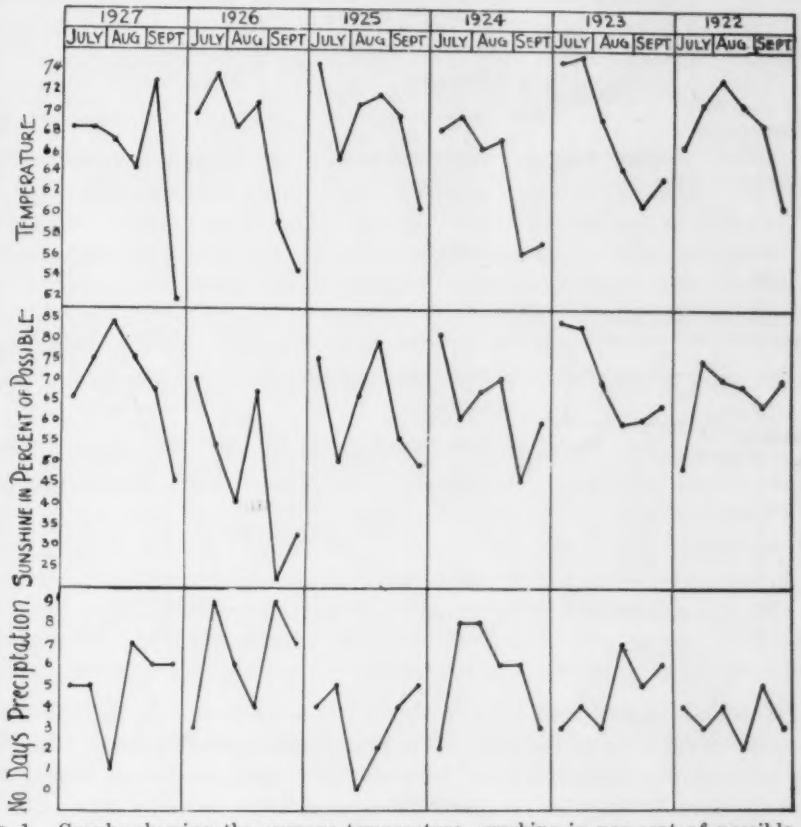

FIG. 1. Graphs showing the average temperature, sunshine in per cent of possible, and number of days on which over 0.01 inches of precipitation occurred, by two-week periods, during July, August, and September, 1922–1927.

month, as determined from the monthly weather reports of the United States Weather Bureau Office at St Paul, Minn.

In table 1 are presented the smut reactions of the same selfed lines of corn which were grown in an artificially induced smut epidemic for two or more years. Notes were taken at three periods of development in 1922 to 1926 (inclusive) and twice in 1926 and 1927. The percentage infection on any particular date was the total amount of infection at that time. For example, midseason infection included early infection as well as infection occurring after the first note was taken. The final note on total smut infection included all infections on the selfed lines. Because of its economic importance, ear smut was calculated separately.

TABLE 1.—*Percentage of total and ear smut infection on selfed lines of corn (selfed 5 years or more) grown in an artificially induced smut epidemic when notes were taken at different periods*

Number selfed lines compared	Early infection						Mid-season infection					
			July 22	Aug. 1	July 20	July 19	Aug. 23	Aug. 17	Aug. 18	Aug. 26	Aug. 21	Aug. 14
	1927	1926	1925	1924	1923	1922	1927	1926	1925	1924	1923	1922
6	—	—	15.3	11.9	11.0	7.3	42.2	17.2	49.7	34.9	51.9	43.4
74	—	—	—	—	—	—	32.4	17.4	—	—	—	—
15	—	—	10.5	6.9	—	—	—	23.1	35.8	26.9	—	—
30	—	—	—	—	—	—	—	19.8	38.1	—	—	—
23	—	—	11.2	8.4	—	—	—	—	38.1	28.1	—	—

Number selfed lines compared	Total infection						Ear infection					
	Oct. 3	Sept. 14	Sept. 10	Sept. 25	Sept. 12	Sept. 8	Oct. 3	Sept. 14	Sept. 10	Sept. 25	Sept. 12	Sept. 8
	1927	1926	1925	1924	1923	1922	1927	1926	1925	1924	1923	1922
6	58.7	46.5	58.9	47.7	56.5	49.4	19.0	16.4	20.5	17.0	15.4	11.0
74	43.9	33.7	—	—	—	—	8.1	7.8	—	—	—	—
15	—	42.2	51.8	28.8	—	—	—	7.7	17.7	7.5	—	—
30	—	40.6	50.6	—	—	—	—	10.0	18.5	—	—	—
23	—	—	51.4	32.7	—	—	—	—	17.0	9.4	—	—

The selfed lines of corn used in this study were grown on the same plot of ground every year of the test. It is possible that conditions for infection were more nearly optimum from 1924 to 1927 (inclusive) than during the first two years of the trial owing to the accumulation of smut in the plot as time went on, although the data obtained do not seem to support this possibility.

It seems, from a study of early, midseason, and total smut infection in table 1 and figure 1 that low precipitation and a high percentage of sunshine, *i.e.*, dry weather conditions, were conducive to a high percentage of smut infected plants. The number of days of precipitation in 1927 and 1925 was low, and the percentage of sunshine was relatively high. A high percentage of smut infection resulted. In 1926 more rainy days were encountered with a lower percentage of sunshine. A lower percentage of smut infection was obtained. In August and early September of 1924, the number of days of precipitation was fairly high with percentage of sunshine slightly lower than in 1925 or 1927, resulting in a lower percentage of smut infected plants.

The correlation between the number of days of precipitation and percentage of ear smut infection, which occurs primarily from August 15 to September 15, is clearly indicated. During the years 1927 and 1925, when the percentage of ear smut was highest, the number of days of precipitation was low and the percentage of sunshine was high. In the years 1926, 1924, and 1923, in which the percentage of ear smut was lower, the reverse conditions were, in general, true.

A significant correlation between temperature and smut infection was not apparent. Temperature did not seem to be a limiting factor in determining the prevalence of smut under the conditions of the experiment. The number of days of precipitation and the amount of sunshine in per cent of possible were of much greater importance than temperature in determining the prevalence of smut in any particular year, in an induced smut epidemic.

Very few data are available regarding infection of corn seedlings in the field with *U. zeae*. Such seedling infection seems to be of comparatively rare occurrence, but when it occurs the seedlings are frequently killed by the smut organism. There is a possibility that the weather conditions earlier in the season may account for this lack of seedling infection. It has been shown that dry weather favors infection of older plants. It is possible that seedlings do not become infected because the weather during May and June is usually not very dry. On the other hand, it is also possible that the inoculum does not accumulate until rather late in the season. Seedling plants may also be less susceptible to natural infection than are older plants.

Observations for a period of years over the state of Minnesota indicate that corn seedlings rarely become infected with *U. zeae*, or at least seldom develop galls. Similar results were obtained, as a result of extensive investigations over a period of five years at University Farm, with corn seedlings grown on smut infected soil. The lack of infection was not due to a scarcity of inoculum, since the soil in which the seedlings were grown

had been in corn continuously for many years. Furthermore, manure inoculated with chlamydospores was applied to the soil each year while the corn was yet in the seedling stage.

In order to obtain information on the relative susceptibility of corn seedlings to *U. zeae*, from 20 to 40 seedlings about 4 to 6 inches high of 34 F_2 recombination lines were hypodermically inoculated with a mixture of sporidial cultures of eight physiologic forms of *U. zeae*. These seedlings were grown in sand in the greenhouse benches. Nearly all of them became infected. Most of the seedlings were killed by the smut. None of the lines was resistant. On July 8, 1927, in a similar manner, 208 seedlings of normal Golden Bantam sweet corn were inoculated in the field, and 182 of them became infected. Of the infected plants, 122 were killed in the seedling stage. On the same day, 234 seedlings, as controls, were injected with a solution in which there were no sporidia. Only one of the controls became infected. About 200 stalks, 2 to 3 feet high, of the same variety, were injected in the same manner as the above controls. Many of these became infected, although this plot was separated from the seedlings by only a few rods. This indicates that inoculum was being blown about. From these results and observations, it is evident that corn seedlings are very susceptible when the inoculum is artificially injected into meristimatic tissue, and that they usually escape infection in the field. Weather conditions during the seedling stage of the corn plant were compared with weather conditions during the later stages of growth for the purpose of learning whether the lack of seedling infection was a result of these conditions.

In table 2 is given the prevailing weather conditions, during the growing seasons from 1923 to 1927 at St. Paul, Minn.

TABLE 2.—*The prevailing weather conditions during the growing season of corn from 1923 to 1927, at St. Paul, Minnesota*

Year	Temperatures (degrees F.)				Precipitation (in inches)				Sunshine (in per cent of possible)			
	June	July	Aug.	Sept.	June	July	Aug.	Sept.	June	July	Aug.	Sept.
1923	70.0	75.2	66.9	62.4	4.28	2.51	1.92	1.10	77	84	65	62
1924	63.6	69.0	67.0	56.7	7.24	1.73	6.51	3.05	63	72	70	53
1925	66.2	70.4	71.3	65.3	5.77	4.28	0.16	3.49	64	63	74	53
1926	63.7	71.8	69.7	57.0	3.65	2.92	4.27	5.43	69	62	66	27
1927	63.6	68.3	65.8	62.4	3.98	2.11	1.95	4.27	67	71	79	58
Av.	65.4	70.9	68.1	60.7	4.98	2.71	2.96	3.57	68	70	71	50

There was considerable fluctuation in temperature, in the amount of precipitation, and in the percentage of sunshine during the month of June.

But in none of the five years recorded was there any appreciable amount of smut on the seedlings, although severe infections developed later in the season. The sunshine in per cent of possible was about the same on the average for June, July and August. The average temperature for June was about five Fahrenheit degrees less than for July, and three degrees less than for August. The precipitation was greater in June than in the two succeeding months. On the average it was decidedly greater than that for either July or August. It seems possible that the excessive rains and the relatively low temperatures in June may act as inhibitory factors in smut development. These two factors, however, do not seem to be the chief factors concerned in the scarcity of seedling infection. In 1926, and in 1927, although considerable corn was planted in the latter part of June or in the early part of July and some was planted in August, yet practically none of the seedlings became infected. Thus it seems that corn seedlings in Minnesota usually escape natural infection in the field. The specific reason for this is not known.

In order to test the comparative resistance of more mature plants under natural infection conditions and when hypodermically inoculated, the same 34 F_2 recombination lines mentioned before were grown in the field. Seedings of the same 34 lines grown in the greenhouse were found to be extremely susceptible when hypodermically inoculated. Plants of these 34 lines, when about 3 feet high, were injected with a conidial suspension of a mixture of eight forms of smut. The results are presented in table 3.

The reactions of these 34 lines of corn when about 3 feet high were quite different from the reaction of the same lines in the seedling stage to the same forms of smut when the plants were hypodermically inoculated. A large number of the lines were highly susceptible when artificially inoculated when about 3 feet high. Some, however, were intermediate in reaction; and a few seemed quite resistant, even when the smut organism was injected directly into the plants.

The correlation between natural infection in a smut epidemic and artificial infection at the location of hypodermic inoculation, of plants of the same lines grown in separate plots, was $+0.40 \pm 0.10$ (Table 3.) This would suggest that there was a certain amount of physiological resistance in these lines to attacks by the smut organism. This type of resistance has been demonstrated by Christensen and Stakman (3). Lines 69, 70, and 75 showed a high degree of resistance to infection both under natural infection conditions in a smut epidemic and when hypodermically inoculated with a mixture of 8 physiologic forms of smut. Lines 48, 68, and 77 were fairly resistant to natural infection but were quite susceptible when hypodermically inoculated. Resistance of these lines to smut infection under normal field conditions may have been largely morphological.

TABLE 3.—*Percentage of smut infection in 34 F_2 lines of corn when naturally inoculated and when artificially inoculated with a suspension of sporidia of 8 physiologic forms of Ustilago zeae*

1927 culture no.	Percentage infected plants		1927 culture no.	Percentage infected plants	
	Natural infection	Hypodermically inoculated in field		Natural infection	Hypodermically inoculated in field
44	25.6	60.0	62	25.0	10.0
45	27.8	45.0	63	26.7	15.0
47	41.3	90.0	64	35.5	0.0
48	2.8	55.0	65	41.9	15.0
49	24.3	85.0	66	33.3	10.0
50	42.6	85.0	67	20.0	10.0
51	32.1	60.0	68	12.5	35.0
52	26.8	65.0	69	18.0	5.0
53	28.8	60.0	70	23.4	5.0
54	55.0	90.0	71	56.9	75.0
55	38.6	100.0	72	30.4	50.0
56	25.0	65.0	73	54.3	85.0
57	27.0	15.0	74	34.0	70.0
58	30.9	5.0	75	18.4	5.0
59	26.9	75.0	76	17.6	35.0
60	30.2	15.0	77	15.7	50.0
61	37.5	25.0	78	28.9	20.0

DISCUSSION AND CONCLUSIONS

From a study of the reactions of selfed lines grown several years on the same plot in a smut epidemic, it could be concluded that low precipitation, high percentage of sunshine, and moderately high temperatures, in other words, dry weather conditions, were most conducive to the production of a high percentage of smutted plants. Temperature was found to be of somewhat less importance in determining the prevalence of smut infection than either number of days of precipitation or sunshine in per cent of possible.

Observations over a period of years indicate that corn seedlings growing in the field rarely become infected with smut, even when the organism is present in abundance. When artificially inoculated in either the greenhouse or field, corn seedlings have proved to be very susceptible to infection. Under normal field conditions in Minnesota these seedlings usually escape infection. The specific reason why corn seedlings escape infection in the field is not definitely known. It seems reasonable to suppose, however, that seedlings must be morphologically or functionally resistant to natural infection in the field. Such resistance is often found among selfed lines of mature corn plants. The location of smut galls on the plants of a

strain is often a strain characteristic. Some lines are highly susceptible to infection at one location on the plants and highly resistant to infections on other parts of the same plants.

F_2 lines from a recombination of 8 selfed lines were grown in a smut epidemic. The same lines were grown in another plot and 20 plants of each line hypodermically inoculated with a mixture of 8 physiologic forms of smut sporidia when the plants were about 3 feet high. The correlation between the percentage of smutted plants in the same lines in these two tests was $+0.40 \pm 0.10$. Certain strains were resistant under field conditions as well as when artificially inoculated. Others were fairly resistant under normal field conditions but quite susceptible when hypodermically inoculated. All of these lines proved to be thoroughly susceptible when artificially inoculated with the same 8 forms in the greenhouse in the seedling stage.

SUMMARY

1. In artificially induced smut epidemics, the effect of environmental factors on the prevalence of infection in selfed lines and crosses of corn was studied. A comparison was also made of the reactions of lines of corn to normal field infection as well as to hypodermic inoculation with the smut-producing organism in the field and in the greenhouse.

2. Dry weather conditions, as expressed by a low number of days of precipitation and a high percentage of sunshine, were found to be conducive to the prevalence of smut. Temperature was not so important a factor as either number of days of precipitation or percentage of sunshine.

3. Corn seedlings proved to be very susceptible when hypodermically inoculated. Infected seedlings are frequently killed by the smut pathogene. Yet observations and experiments over a period of years indicate that seedling infections with gall formation in the field are of comparatively rare occurrence. The specific reason for this is not known. Environmental factors do not satisfactorily explain this lack of infection. It seems possible that corn seedlings are morphologically or functionally resistant to natural field infection.

4. A correlation of $+0.40 \pm 0.10$ was found between natural infection in a smut epidemic and artificial infection of the same strains of corn by hypodermic inoculation with a mixture of 8 physiologic forms of smut.

5. Some lines of corn were resistant to smut infection under natural conditions as well as when hypodermically inoculated with a mixture of sporidia of 8 forms of smut, when the plants were 3 feet high. Other lines were resistant to natural infection but susceptible when hypodermically inoculated.

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DETERMINATION OF LOSSES DUE TO SMUT INFECTIONS IN SELFED LINES OF CORN[1, 2]

F. R. Immer and J. J. Christensen.[3]

The annual loss of corn due to smut, *Ustilago zeae* (Beckm.) Unger, for the entire United States (1922–1925) is estimated at 1.7 per cent of the total crop, or more than 47,000,000 bushels. Under some conditions this loss may be much higher. Losses ranging from 5 to 10 per cent are frequently reported from individual states (7).

Very few data are available on the amount of loss caused by smut infections on individual corn plants. Hitchcock and Norton (4), in 1896, found that in a badly smutted field the infected plants produced one-third less corn than the smut-free plants in the same row. In their study all plants with a trace of smut anywhere on the plant were classed as infected. Clinton (1), in 1900, found that smutted plants produced 8 per cent fewer ears than smut-free plants. Hayes, Stakman, Griffee, and Christensen (3) studied the effect of ear smut on yield. They found a low negative correlation between the percentage of ear smut and yield index of selfed lines grown in a smut epidemic. The loss in some F_1 varietal crosses grown under normal field conditions varied from 1.4 to 8.8 per cent of the ears. Later, Hayes (2) studied smut reaction and yielding ability of selfed lines which were grown under ordinary field conditions. Very susceptible lines had been discarded before the study was made. There was a low negative correlation between yield and percentage of smutted plants in these selfed lines.

The purpose of the present study was to determine the effect of smut galls of different sizes and in different locations on the plants in reducing yield in selfed lines of corn.

METHODS AND MATERIALS

This study was made on pairs of plants of the same selfed lines of corn growing one foot apart and subjected to the same amount of competition

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[2] This study was made as a part of the general program of breeding for disease resistance being conducted cooperatively by the sections of Plant Genetics and Plant Pathology.

[3] The writers wish to express their appreciation to Drs. H. K. Hayes and E. C. Stakman for helpful suggestions and criticisms while conducting this study.

TABLE 1.—*Extent of reduction in yield of shelled corn from plants of selfed lines of corn (selfed 5 years or more) due to smut galls of different sizes and at different locations on the plants*

Location and size of galls	No. of paired comparisons	Mean yield of plants in grams		Percentage reduction in yield due to smut	Odds that smut reduced yield
		Smut free	Smutted		
First shoot above ground	15	55.03	37.77	31	29.1:1
Second and higher shoots above ground	18	50.50	36.81	27	38.2:1
Total infection below ear	36	52.82	34.14	35	1,266.0:1
Neck infection	20	59.40	27.65	54	>10,000:1
Total infection above ear	35	57.04	28.11	51	>10,000:1
Large infections below ear	14	50.21	23.71	53	4,990.0:1
Medium infections below ear	18	53.81	39.51	27	12.6:1
Small infections below ear	4	57.50	47.38	18	2.5:1
Large infections above ear	15	56.43	3.20	94	>10,000:1
Medium infections above ear	12	50.25	35.54	29	25.3:1
Small infections above ear	8	68.38	63.69	7	3.7:1

from surrounding plants. Each pair consisted of one plant with a single smut infection and another which was free from smut. An effort was made to select pairs of plants in which the only difference seemed to be the fact that one plant was smutted and the other was not. Notes were taken on the size and location of smut galls on the infected plants. The smut galls were classified as small, medium, or large, depending on size. Galls designated as small were less than 1½ inches in diameter. Those designated as large were at least 4 inches in diameter, while those designated as medium were intermediate between small and large. Neck infection referred to galls on the upper node or internode, *i.e.*, just beneath the tassel. Infections of the rudimentary ear buds were classed as shoot infections.

The ears of both plants of each pair were numbered, harvested, and dried to approximately 3 per cent moisture; then the yield of the shelled corn from

each plant was determined. The pairing method was used to determine the reduction in yield due to smut infection by determining the difference in yields between each pair of selected plants. The significance of this reduction in yield was determined by the use of "Student's" tables (5, 6).

EXPERIMENTAL RESULTS

In table 1 are presented the data on the reduction in yield of shelled corn, due to smut infections, on plants of selfed lines of corn. Each smutted plant was compared directly with a non-smutted plant of similar breeding, growing at a distance of one foot.

With the refined methods of analysis used in this study, valid conclusions may be drawn from a comparatively limited amount of material. In table 1 it is clearly shown that reduction in yield of corn is influenced markedly by the size of the smut infections. Infections above the ear reduced yield more than did smut galls below the ear. It might be noted in passing that smut infections below the ear are very largely rudimentary ear bud or shoot infections while infections above the ear are direct nodal or internodal infections. There seems to have been little difference in reduction in yield due to smut galls at the first shoot above the ground and those higher on the stalk.

The reduction in yield (54 per cent) due to smut galls located on the neck of the plants was rather striking. The odds were found to be very great, so that this reduction in yield is not due to chance.

DISCUSSION AND CONCLUSIONS

From the data obtained in the present study it seems likely that the losses caused by corn smut have been underestimated. It is relatively easy to appreciate the losses caused when seedlings are killed, when ears are destroyed, and when stalks are badly distorted. But there has been only meager information regarding the reduction in yield caused by smut galls on otherwise apparently healthy plants. It is evident from the results presented in this paper that the yield of shelled corn is reduced materially when there are smut galls of medium or large size on the plants. It is somewhat surprising that yields are reduced more when the galls are above the ear than when they are below it: 94 per cent as compared with 53 per cent in the case of large galls. There is a positive correlation between size of gall and reduction in yield, which, of course, would be expected.

The methods used in this study make possible an accurate determination of the extent that various types of smut reaction reduce yield. If normal varieties are as seriously injured by smut as selfed lines, and if similar results are obtained in other localities, it will be necessary to modify our ideas regarding the destructiveness of corn smut.

SUMMARY

1. A study was made to determine the effect of smut galls of different sizes and of different locations on the plant in reducing yields.
2 Reduction in yield of shelled corn was influenced markedly by the size of smut galls. The larger the galls on the stalks, the greater was the reduction in yield. Infections above the ear reduced the yield significantly more than did galls below the ear.

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THE REACTIONS OF PLANT STEMS TO FUNGOUS PRODUCTS[1]

C. R. Hursh

INTRODUCTION

The studies of Haskell (4), Brandes (3), and Bisby (2) served to arouse an increased interest in the phenomenon of wilting of plants attacked by vascular parasites. These investigations demonstrated that green plant stems wilt when the basal ends are cut and placed in liquids in which parasitic fungi have been cultured. The problem then presented itself as to the cause of this induced wilting and the significance of such experiments in a study of wilt diseases. It was postulated that the pathologic condition in plants infected with certain wilt diseases might be brought about by some fungous products deleterious to the host tissue that had been transported considerable distances through the stem from the point of the fungous attack. Should the presence of such substances actually be demonstrated, it would be an important contribution to an understanding of the physiology of fungous parasitism. A number of papers have appeared in which the subject is considered. This literature has been most recently reviewed by White (8) and Rosen (7).

The conclusions of different investigators regarding wilting phenomena are not all in agreement. The fact that plant tissue is disintegrated by certain fungous filtrates [as first shown by DeBary (1)] has frequently been given only secondary consideration by some investigators in their search for substances particularly active in producing the death of plant cells and at the same time readily transported through the water-conducting system of the plant stem. The methods used have been so different that it is difficult to correlate the results that have been reported. This is decidedly true when the degree and rapidity of wilting of plant stems has been used as the principal test for the injurious fungous products. The writer has had occasion to repeat many of the investigations on the induced wilting of plant stems and to make comparative tests with filtrates and isolated products from cultures of a considerable number of fungi. The present discussion concerns itself with certain methods employed and the validity of the results obtained by these methods. The nature and specificity of fungous products and the methods of culturing fungi for study of their products are not considered here.

[1] This discussion is from a report on investigations of methods of attack for the study of disease resistance in plants, conducted under Rockefeller Foundation for International Education and National Research Council Fellowships.

WILTING OF CUT STEMS

The disintegration of tissue discs has proved to be a useful criterion for the injury that a fungous filtrate may produce upon plant tissues. This method is adaptable to standardization. By contrast to the tissue disc method, the wilting of cut plant stems when placed in fungous filtrates presents many discouraging and questionable features as a test for injurious fungous products.

The effect of age of stem.—The age and condition of the plant stems are factors that greatly influence their wilting response when placed in fungous filtrates. It is a simple procedure to demonstrate that rapidly growing, succulent stems will wilt more quickly than older stems or stems grown under hardening conditions.

Table 1 illustrates the difference in behavior that can be secured by using stems of different ages and conditions in the same fungous filtrate. In this experiment, filtrates from two fungous strains proved to be pathogenic to tomatoes were used, together with a filtrate from *Aspergillus niger*. The cut stems were placed in sterile filtrates from three-weeks-old cultures on a modified Richards' solution. The filtrates were diluted with three volumes of distilled water. A selected variety of Stone tomato was used, although entirely similar results may be secured with other varieties of tomato, and with other plant species.

TABLE 1.—*Relation of age and condition of tomato stem to wilting when placed in fungous filtrate*

Condition of stem	Degree of wilting and leaf injury after 12 hours in filtrate from			
	Sterile culture medium	*Fusarium lycopersici*	*Verticillium albo-atrum*	*Aspergillus niger*
Forced seedling 6 inches high	none	severe	severe	severe
Hardened seedling 6 inches high	do	moderate	moderate	moderate
Normal stem 12 inches high	do	none	none	none
Mature stems	do	do	do	do
Growing tip of mature stem	do	severe	severe	severe

Wilting occurs only in the growing, succulent stems. The leaves on the mature stems do not wilt in 12 hours under the conditions of this experiment, but vascular discoloration toward the cut end of the stem does occur. In the succulent stems this discoloration is less evident or entirely lacking.

From the collapse of the basal cells of the succulent stems it is evident that there are substances present in the fungous filtrate that are injurious

to plant cells. The fact that the leaves of the mature stems do not wilt may indicate that the injurious substances are carried upward through the vascular system and subsequently deposited or otherwise rendered inactive before they reach the cells of the leaf tissue. Associated with this inactivation there occurs the discoloration of the vascular system and an interference with the transpiration stream. A consequent pathogenic condition will then manifest itself by the wilting of the upper plant stems.

From the data presented in table 1 it is obvious that degree and rapidity of wilting are not adequate for determining the injury by fungous filtrates to plant stems. Observations must be made on the nature of the actual injury that results. In some cases there is a rapid collapse of the cells at the cut surface. The transpiration stream is stopped, and the consequent wilting of the upper leaves takes place almost as rapidly as though the cut stem had been allowed to wilt normally without contact with any liquid whatever. If such a stem is placed in fresh water after recutting the basal end it may be expected to recover, as previously pointed out by the writer (6). It has also been shown that succulent stems kept in fresh water or in dilute salt or sugar solutions for 24 hours previous to being placed in the fungous filtrates show a different reaction from stems cut and placed in the fungous filtrate at once.

Rapid and gradual wilting.—The phenomenon of rapid wilting and recovery occurs in succulent stems and at higher temperatures. As the wilting that has attracted the attention of certain investigators has been the rapid wilting of succulent stems, the question as to whether or not the stems will recover is a significant one. It is important in all cases of rapid wilting to remove a number of plants before they become dry and to test their recovery in fresh water after a portion of the stem has been cut away at the basal end to insure a freshly cut surface exposed to the liquid.

Besides rapid wilting, a gradual wilting of plant stems may be brought about in fungous filtrates. A considerable time is required before the stem is completely wilted. This type of wilting is more frequent in woody stems. Yellowing may occur, accompanied by vascular discoloration. Rapid recovery in fresh water has not been observed.

In the two types of wilting contrasted above, both of which can be brought about by the same filtrate, the difference seems to be partly in the nature of the water-conducting cells of succulent and woody stems. In rapid wilting one asks whether or not the filtrate is actually being carried into the stem. The gross wilting phenomenon is therefore not so important as is the effect of the injurious substances on certain localized cells or tissues in the stem.

VARIATIONS IN PLANT REACTIONS

Many different types of wilting and other responses may be described for plant stems placed in fungous filtrates or in any solutions containing substances injurious to plant cells. Some substances are carried through the stem and kill small, irregular patches of leaf tissue with little injury to the rest of the plant. Other substances blacken the leaf veins, either with or without producing wilting of the entire stem (see fig. 1). Many necrotic conditions of plant tissue that may be brought about in the laboratory by products isolated from fungous filtrates do not resemble any

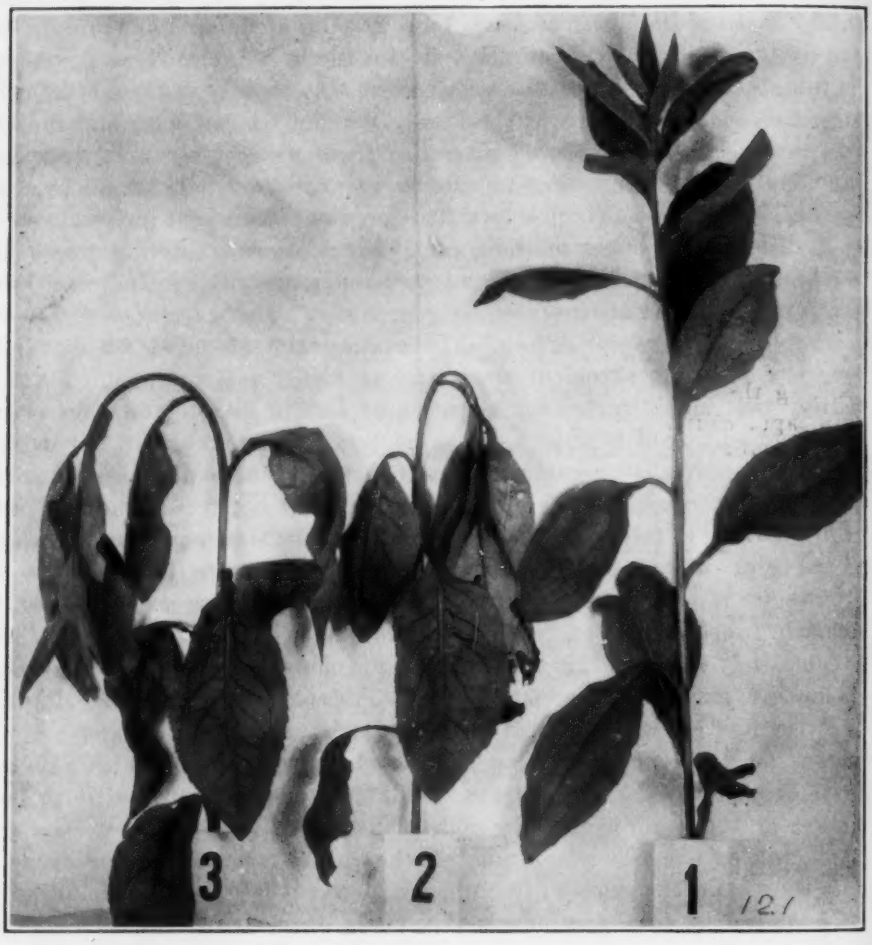

FIG. 1. Solidago plants showing injury from products of *Fusarium oxysporum* Schl. This injury is not analogous to pathologic conditions appearing in the field. Note the darkening along the veins, while the remainder of the leaf is still green.

pathologic conditions observed in diseased plants in the field. For example, the necrotic injury to plant stems that can easily be induced in the laboratory by salts such as nitrites may have no direct analogy to pathologic conditions occurring in the plants attacked by wilt-producing fungi. Figure 1 illustrates a typical salt injury that can be secured in the laboratory by products isolated from fungous filtrates where the medium is of the nature of Richards' solution. This type of injury is not recognized as a common pathologic condition brought about by parasitic fungi. This lack of correlation between wilting of stems in the laboratory and in the field emphasizes the importance of studying particular plant tissues that have been affected. For this study, observations with plant stems placed in fungous filtrates are useful in comparing the effect of known and unknown substances upon the vascular system of the plant. The observation should be of vascular discoloration and development of pectic or other hydrophyllic substances produced by plant tissues in response to stimulation.

TEMPORARY WILTING

In the well-known wilt diseases produced by species of *Fusarium* and *Verticillium* it is a matter of common observation that, where woody stems are concerned, dry, hot, windy days will produce wilting in attacked plants to a greater degree than in unattacked plants. But even the attacked plants recover their turgescence when the conditions of excessive transpiration are removed. This type of wilting may be different from the final wilting of attacked plants in the field, but it suggests that the difference may be one of degree only.

The fact that attacked plants may recover from the temporary wilting indicates that the condition is due to some interference with the water absorbing and conducting systems of the wilted plants, rather than to any poisoning of the upper leaves by fungous products. When the stem at the point of attack has reached advanced stages of disintegration, the products of the dead cells may themselves be as much responsible as are the fungous products for the pathologic condition of the upper stem. The final wilting and dying occur only after considerable functioning tissue of the plant has been destroyed by the pathogen.

With a few questionable cases excepted, the pathology of tree diseases does not indicate that fungous products are transported very far from the point of attack of the fungus. As a rule, a complete girdling of seriously injured cambium is necessary before the upper limb manifests pathologic conditions.

The common Botrytis wilt of peony is not a vascular disease, but it illustrates a point in the wilting of diseased plants. The roots of diseased

plants are not affected. The wilting is rapid after it once begins, requiring only one or two days for the stem to become dry. There is little or no yellowing prior to the appearance of the wilt. If the individual stems in the clump are observed daily, it will be seen that the Botrytis lesions appear at the base of the stem and develop to a considerable extent with no symptoms of injury appearing in the leaves. As soon as the lesion completely encircles the stem the leaves will begin to wilt. On the first day after the symptoms of wilting appear if the stems are cut with a sharp knife a short distance above the lesion and placed in fresh water, the leaves will return to their normal turgid condition and will remain in this state as long as will normal, unattacked stems. Table 2 gives the result of observations.

TABLE 2.—*Recovery of peony stems from wilt after removal from the diseased plant to fresh water at 20° C.*

Condition of stem when cut	Hours elapsed after wilt first observed	Condition of stem after 2 hours in fresh water	Condition of stem after 24 hours in fresh water
Sound	—	turgid	turgid
Flaccid, beginning to wilt	6	do	do
Badly wilted, not drying	18	recovering	do
Drying	30	drying	dead

These observations indicate that wilting has been due to interference with the water conducting system of the plant, and that the upper stem and leaves have not been seriously injured by substances given off by the fungus. It is known that *Botrytis* produces organic acids in culture and that these acids are particularly injurious to plant tissue. However, the wilting of the upper peony stems as observed in the field evidently is not due to organic acids that have been transported through the stem. At the same time, there is good evidence that the killing of plant cells at the point of lesion is directly due to organic acids. Higgins (5) has recently reported this to be the case in the parasitism of *Sclerotium rolfsii* Sacc., and has given an historical review of the subject. A more extensive literature review is that by Zimmerman (9).

DISCUSSION

In attempting to correlate laboratory studies with pathologic conditions occurring in the field, it appears that wilting is not evidence of the movement of highly injurious fungous products through the stem from the point of attack of the pathogen to the upper leaf tissue of the host. Specific fungous products, such as enzymes and acids, appear to be injurious chiefly to the plant cells that are in close proximity to the attacked

tissue. Where the water conducting system is parasitized or is in direct contact with attacked plant tissues, it would be reasonable to expect that certain fungous products might be transported through the transpiration stream to the leaf tissue. If the transported fungous products are highly injurious, it would be expected that a necrotic injury would result in the leaf tissue. But such is not the pathologic condition of the leaf tissue of plants attacked by the common wilt-producing fungi. The symptoms are a general wilting and debility, suggesting starvation and lack of sufficient moisture from the transpiration stream. The abnormal functioning of the vascular system itself may be induced through noxious substances produced either by the pathogen directly or through the disintegration of the host cells. Observations do not seem to support the hypothesis that pathologic conditions such as yellowing and wilting are due to fungous products directly transported to the leaves.

The evidence that wilting in plant stems may not be due to transported fungous products makes it probable that wilting response alone is not the most desirable criterion of the effects of these products upon plant tissues. Further study of fungous products is nevertheless imperative. Aside from the wilting of stems there are a number of available methods for the study of the effects of fungous products on plants. As an example, for soil inhabiting organisms, the effects of the products of these fungi upon root establishment, root hair development, seed germination, etc., are all fertile fields for investigation. It is a simple procedure to demonstrate that a comparatively dilute filtrate from different species of *Fusarium* will inhibit root development. This reaction may be produced by placing mature tomato stems, showing indications of adventitious roots along the internodes, into large test-tubes containing the fungous filtrates sufficiently diluted to produce no wilting of the leaves. The controls are placed in water or in the uninoculated culture media. The roots on the control stems will begin to develop at once, while those on the stems in the fungous filtrate will develop very slowly or not at all. This particular test is adaptable to studies with entire plants. Injection of sterile filtrates into the cavities of hollow stems and leaves has some possibilities for adaptation to experimental studies. Direct observation of individual cells, root hairs, etc., is even more promising of results. Particularly is the significance of vascular discoloration one of the most desirable focal points for study of the pathology of vascular diseases.

SUMMARY

The reaction of plant stems to fungous filtrates indicates an interference with the normal functioning of the water-conducting system.

Wilting is not, in itself, an adequate criterion for the injurious character of fungous products to plant tissues. The age and condition of the stems, the length of time elasped after cutting, the treatment to which they are subjected during this period, all influence the rapidity and degree of wilting that will occur when stems are placed in fungous filtrates.

A better understanding of the nature of these reactions is dependent upon further study of the particular cells and tissues that are directly influenced by the injurious substances present in fungous filtrates.

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A POWDERY MILDEW PARASITIZING CHINESE CABBAGE

W. H. Davis

A powdery mildew was observed on the leaves and floral parts of Chinese cabbage plants (*Brassica pekinensis* Rupr.) which were growing in vegetable gardens at Amherst, Massachusetts. As no description of this disease and organism on Chinese cabbage was found in our most available phytopathological literature, it seemed advisable to solve the following problems. (1) How severe is the disease and what are its symptoms? (2) Does this organism parasitize other crucifers which are cultured in the vicinity and each of which is parasitized by a powdery mildew? (3) What is the genus and species of the organism?

The mildew was first noticed on September 15, 1925. After this date fortnightly observations were made until November when some of the diseased plants were removed from the gardens, transplanted in pots and cultured in the greenhouse for investigation during the following winter (Pl. XIII, B). However, the disease organism reappeared on other Chinese cabbage plants cultured in the same gardens during the autumns of 1926 and 1927. Some of these diseased plants were also potted and cultured in the greenhouse, as was done in 1925.

The disease first appeared on the leaves of Chinese cabbage as small, puckered, wrinkled areas delimited by the veinlets (Pl. XIII, B, 2–4). About one week later, the uppermost surfaces of these areas turned a yellow-orange or bronze color and the leaf appeared as if infected with a virus disease. Finally, the whole leaf turned yellow and many dead areas of a tan color were scattered over its surface. Later, the whole leaf turned a tan color, died, and was easily severed from the stem (Pl. XIII, B, 1). However, a fine white powdery substance, the conidia and mycelium of the organism, could generally be observed on the upper surfaces of the leaves before the yellow puckered areas appeared. The mycelium on the surface of the leaf was persistent and sub-pannose, but the conidia were comparatively few in number (Pl. XIII, B, 1 and 4). The yellowing and dying of small areas on the facies of the leaves were the most prevalent early symptoms of the disease. However, the organism attacked both the upper and the under surfaces of some leaves and generally rendered them nonusable.

In the gardens, the most damage occurred to the flower stalks (bracts) and pods (siliques) which turned brown and died prematurely. In gen-

eral, the damage was very slight to those leaves which had matured before the organism appeared in the autumn. Two cases were observed where it was believed that the mildew killed the plant. In the greenhouse the injury to the plants was more severe than in the garden, where diseased plants were seldom killed.

The inoculations were performed with potted plants cultured in a damp chamber at 15° C. Small portions of the leaves bearing the organism were laid on the surfaces and in contact with healthy ones. Each inoculation was repeated at least three times and some of them twelve times. The following plants were inoculated with viable conidia from Chinese cabbage (Wong bok): common cabbage (*Brassica oleracea* var. *capitata* L.),[1] Danish Ballhead variety; turnip (*Brassica rapa* L.), Burpee's White Egg; radish (*Raphanus sativus* L.), French Breakfast; Chinese cabbage (*Brassica pekinensis* Rupr.), Wong bok: also plantain (*Plantago major* L.), broad leaf plantain—with green leaf bases; cucumber (*Cucumeris sativus* L.), Davis' Perfect; lettuce (*Lactuca sativa* var. *capitata* L.), New York Head; bean (*Phaseolus vulgaris* var. *humili* Alef.), Golden Wax.

The final results of these inoculations showed that conidia from Chinese cabbage infected common cabbage (Pl. XIII, A), turnip, Chinese cabbage, and radish. All other inoculated plants remained healthy except one series in which three inoculated plantains were infected. Two weeks previous to these inoculations in January, six plantains were removed from the field, potted, and cultured in the greenhouse. All the plants being healthy, three were inoculated with conidia from Chinese cabbage and three reserved as checks. The checks remained healthy while two of the inoculated plants showed infection ten days later. Likewise, 12 subsequent inoculations of other plantains were made and all the plants remained healthy; so it was concluded that infection in the first inoculation was due to some contamination and that this mildew on Chinese cabbage seldom, if ever, infects broad leaf plantain.

Within a radius of one-half mile from the gardens, in which these infected Chinese cabbage plants were growing, a powdery mildew was observed on each of several other plants: turnips, beans, cucumbers, and broad leaf plantain. Chinese cabbage plants were inoculated with viable conidia from each of these plants, and conidia from turnips produced the only infection.

Infected plants of Chinese cabbage, common cabbage, and turnip were under constant observation for the purpose of detecting perithecia or a perfect stage of the fungus. What appeared to be immature or abortive perithecia of the Erysiphe type were observed on the midribs and leaf bases

[1] Latin binomials as listed by BAILEY, L. H. Manual of Cultivated Plants, 1924.

of old leaves from Chinese cabbage and common cabbage, but mature perithecia containing asci with ascospores were not detected. Diseased plants of Chinese cabbage, turnip, and common cabbage were also subjected to various conditions of freezing and thawing under controlled laboratory conditions and under outdoor conditions. Furthermore, diseased plants were under observation in the field and in the greenhouse for two years, but no mature perithecia were observed. In this respect the writer's knowledge of the perfect stage of this fungus is similar to Salmon's statement[2] concerning *Erysiphe ploygoni* D. C. "No one—knows—what other form it takes or how it hibernates through the winter."

Examination of the organism causing the powdery mildew on Chinese cabbage showed that the conidia were borne singly on the conidiophores (Fig. 1, No. 1, 2, 3). Conidia are cylindrical-elliptical, the ends are rounded and they are not constricted (Fig. 2, No. 1–16). Measurements: limits, 12–18 × 28–52 microns; standard, 14 × 42 microns (100 measured); conidiophores averaged 9 × 58 microns and were composed of 2 to 3 cells (Fig. 2, No. 17, 18, 19); hyphae, 6 microns in diameter (Fig. 1, No. 20).

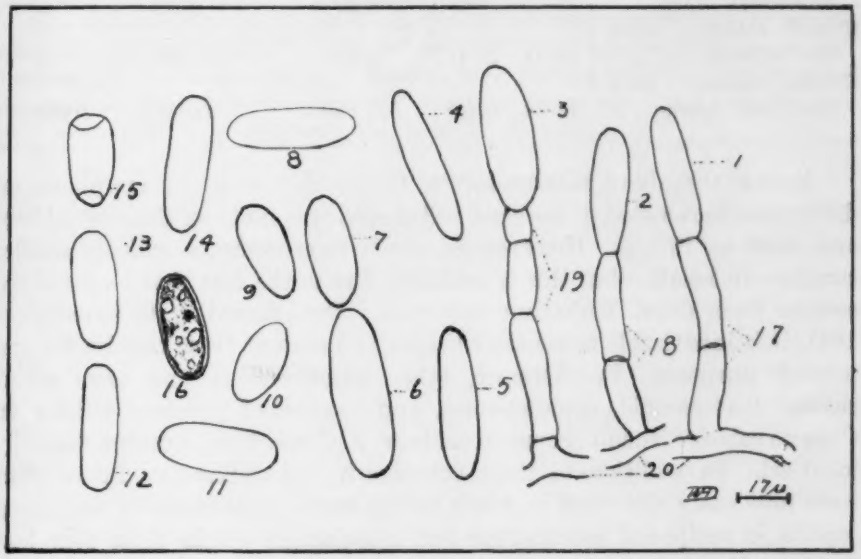

FIG. 1. Conidia and conidiophores of *Erysiphe polygoni* D. C. from leaves of Chinese cabbage. Drawings outlined by aid of a camera lucida. 1–16. Subcylindrical conidia with rounded, unconstricted ends and of various sizes; 1, 2, 3. Conidia borne singly on conidiophores; 15. A non-viable, air-dried, conidium; 16. Thin wall and granular content of a viable conidium; 17, 18. Conidiophores composed of two cells; 19. Of three cells; 20. Hyphae of the fungus.

[2] SALMON, E. S. A monograph of the Erysiphaceae. Memoirs Torrey Bot. Club 9: 174–191. 1900.

In table 1 are given measurements in microns of 25 viable conidia and conidiophores of mildew removed from the following plants and mounted in water under cover slips: (*1*) a turnip which had been inoculated with conidia from Chinese cabbage; (*2*) naturally infected Chinese cabbage plants; (*3*) a Chinese cabbage plant artificially inoculated with conidia from turnip. Salmon's measurements for the mildew on turnip are included.

TABLE 1.—*Measurements in microns of 25 viable conidia and conidiophores of mildew from four different sources*

Host	Conidium measurements in microns		Conidiophore measurements in microns	
	Limits	Standards	Standards	Cells
Turnip inoculated from Chinese cabbage	11–18 × 30–49	14 × 40	10 × 60	2–3
Turnip (Salmon's measurements)	13–15 × 30–40	Not given	Not given	Not given
Chinese cabbage, naturally infected	12–18 × 29–47	14 × 41	9 × 58	2–3
Chinese cabbage inoculated from turnip	13–18 × 32–49	15 × 40	9 × 60	2–3

Among the above measurements, no decided constant morphological differences were noted in the sizes and shapes of conidia on Chinese cabbage and those on turnip. However, Salmon's measurements indicate smaller conidia—in length—but this is probably due to the fact that he measured conidia from dried, herbarium materials. But, according to Salmon (p. 184), it is inadvisable to consider shapes and sizes of fresh conidia for systematic purposes. Furthermore, other measurements and observations showed that conidia, conidiophores, and hyphae of powdery mildew on Chinese cabbage, turnip, common cabbage, and radish were morphologically identical. In addition to these comparative measurements, inoculations were previously described in which infections were obtained by employing conidia in reciprocal inoculations and inoculations within these four host species. For these reasons, the mildew on Chinese cabbage, turnip, common cabbage, and radish was considered one morphological and physiological species which compares favorably with that reported on turnip by Salmon as *Erysiphe polygoni* D. C. However, physiological forms of this mildew might have parasitized other hosts but this phase of the problem was not investigated.

Finally, the powdery mildew on Chinese cabbage may not be considered a severe disease in this climate. It first appeared in the autumn and caused a yellowing, curling, and dying of the leaves and floral parts. The mildew infected other crucifers, among them turnip, cabbage, and radish, but normal perithecia were not observed on these hosts under the conditions to which they were exposed. The fungus compares favorably with *Erysiphe polygoni* D. C., reported on turnip in Europe by Salmon. It is suggested that this Latin binomial be used for its classification.

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EXPLANATION OF PLATE

PLATE XIII

Photographs of potted plants infected with *Erysiphe polygoni* D. C.

A. An infected common cabbage plant which had been artificially inoculated with conidia from a Chinese cabbage (B). 1, 2, 3, 4. Infected leaves. The mycelium is non-persistent on these leaf margins but pannose on the lower, viable green leaves 1, 2, 4.

B. A naturally infected Chinese cabbage which had been removed from the garden, potted and cultured in the greenhouse for one month. 1. Persistent mycelium on half of a dead leaf; also shown at 4. 2–4. Infected leaves showing symptoms of the disease; puckered areas which have the appearance of mosaic. Many leaf traces show the number of leaves which have apparently been killed by the fungus.

B
A

A FIELD METHOD OF INSURING POSITIVE ATTACK WITH SOME CEREAL DISEASES[1]

W. W. MACKIE

For many years the writer has been working to breed rust-resistant cereals in California. Stem rust, for instance, under California conditions causes severe injury over the entire state at irregular periods, and in certain localities is the limiting factor in the production of wheat. To test the resistance of hybrid wheat plants in the F_1, F_2 and later generations, in order to make early separation of resistant hybrid plants or fixed varieties, it was necessary to produce artificially annual attacks of epidemic severity. The method here presented has so successfully met the conditions in California that the writer hopes it may prove useful to breeders of cereals in other regions where conditions are similar.

Greenhouse inoculations for diseases attacking the leaves, it is true, will indicate, in the seedling stage, the mode of inheritance. In the cases of stem rust[2], leaf rust[3], and mildew[4], several strains or physiologic forms of the organism may be involved in the field attack, while one or more forms have been used in greenhouse inoculations. Identification of physiologic forms of stem rust collected on the University Farm at Davis, California, during a number of years, has shown that the same forms usually occur from year to year. The use of the field attack or epidemic appears workable and practicable for stem rust. It is probably, though not positively, proved that the same conditions would hold for the other cereal disease-producing fungi which possess more than one physiologic form.

Creating an artificial epidemic in the field always has been difficult. It has been observed frequently that wheat plants artificially inoculated with stem rust on the first seedling leaf do not show the same qualities of resistance found later as the plants near maturity.

The writer has observed that many cereal diseases, including leaf rust, stem rust, stripe rust of wheat, and powdery mildew of wheat, oats, and

[1] Investigations conducted cooperatively between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the California Agricultural Experiment Station.

[2] STAKMAN, E. C., and M. N. LEVINE. The determination of biologic forms of *Puccinia graminis* on *Triticum spp.* Minn. Agr. Exp. Sta. Tech. Bul. 8. 1922.

[3] MAINS, E. B., and H. S. JACKSON. Physiological specialization in the leaf rust of wheat, *Puccinia triticina*. PHYTOPATH. **16**: 89–120. 1926.

[4] REED, G. M. Varietal resistance and susceptibility of oats to powdery mildew, crown rust and smuts. Mo. Agr. Exp. Sta. Res. Bul. 37. 1920.

barley, halo blight of oats, and barley scald, cause little or no damage until the plants tiller or are in head. Stem rust especially causes its worst damage after the plants are in full head, usually in the milk or soft-dough stage.

In California, the dry season is near at hand when cereal plants are heading. Usually no considerable quantity of rain falls after this time. Increasing heat and drying north winds frequently aid in restricting the progress of cereal diseases of the rust type so that no real indication of the inheritance of resistance can be obtained. A period of two or more such years may occur in sequence. In the meantime the crops of hybrid cereals have been carried through the F_1 and F_2 or even the F_3 generations and beyond the possibility of determining the Mendelian inheritance of disease resistance. The certainty and comparative rapidity which follow the breeding of varieties of wheat resistant to bunt, as compared with the uncertainty and indefinite results of breeding varieties of wheat resistant to stem rust illustrate this point. In the case of bunt, positive infection of a relatively high percentage of the possible number of susceptible plants is secured by the usual methods of artificial inoculation of the seed. No such certainty occurs in the usual field methods employed to induce stem-rust infection.

ARTIFICIALLY INDUCED STEM-RUST ATTACKS

Various devices have been employed to induce stem-rust epidemics in the field. Barberry bushes carrying stem rust have been planted as a hedge surrounding the plats of cereals under test. Sprays containing rust spores have been applied at the critical periods. The writer has applied irrigation water repeatedly at the favorable dates for stem-rust attack. All of these methods, so far as observed, have failed to give a real epidemic, unless the general climatic conditions were favorable, as measured by the stem-rust attack under natural conditions. A satisfactory method of artificially creating an epidemic of stem rust and several other cereal diseases has been discovered and perfected in experiments made by the writer on the University Farm at Davis, California.

FALL DISEASE ATTACKS FOLLOWING JULY SOWING

In breeding for agronomic characters in cereals, it is frequently desirable to hasten the process by growing more than one generation during the year. For years past, the writer has secured a second generation of wheat, barley, and oats in the same year at Davis, California, by sowing seed from the regular June harvest in the July immediately following. Although no rains fall during the summer, these cereals may be germi-

TABLE 1—*Relation of weather conditions to stem-rust and other cereal-disease epidemics at Davis, California*

	Temperatures in degrees F					Relative humidity in per cent			Rainfall in inches			Cloudiness		Sunshine	
	Mean	Departure from normal	Highest	Lowest	Greatest daily range	5 a. m.	5 p. m.	Mean	Total	Departure from normal	No. rainy days	No. Clear days	No. partly cloudy days	Actual No. of hours	Per cent of possible
March	64.4	+6.1	82	43	31	80	49	64	0.36	−0.62	2	27	1	344	93
April	63.0	+4.9	91	46	29	87	58	72	4.25	+2.25	7	17	5	282	70
May	64.4	+6.1	98	43	31	83	38	60	0.50	−2.96	1	25	6	399	89
September	67.5	−1.8	97	47	42	74	30	52	tr	−0.39	0	29	1	354	95
October	64.7	+1.8	87	45	32	76	44	60	2.14	+1.10	4	26	3	302	87
November	58.6	+5.0	78	41	30	81	59	70	4.48	+2.33	11	10	9	144	47

nated, and grown to successful maturity late in November, by means of irrigation. They are then harvested and, after a period of drying, sown for the regular harvest in the next June. At the November harvest many cereal diseases common to the region appeared in epidemic severity.

By sowing both the resistant and susceptible varieties involved in the crosses bred for the genetical study of resistance to cereal diseases, a remarkably certain and positive record of inheritance of disease resistance has been obtained for several successive years.

RELATIONS OF CLIMATIC CONDITIONS TO THE ATTACKS OF STEM RUST AND SOME OTHER CEREAL DISEASES

The spring weather of 1926 over the whole western part of North America, including California, greatly favored the development and spread of stem-rust attacks. A severe epidemic throughout the West Coast States followed, as shown in table 1. The weather data for Sacramento, California, compiled by the United States Weather Bureau, indicates a combination of weather conditions favorable for the development of cereal diseases.

The months of March, April, and May, covering the period in which cereal disease attacks are most pronounced, are compared with September, October, and November, the fall months in which epidemic attacks of cereal diseases were induced by artificial cultural methods. May and September, April and October, and March and November are the comparable months. In temperature, hours of sunshine, and rainfall, the climatic conditions for these paired months closely resemble one another. For humidity and relation of temperatures and humidity to maturity, the changes occur in an inverse ratio for the two periods. In the spring, the temperature increases and the humidity decreases with the advance of the season, but in the fall the temperature decreases and the humidity increases coincidently with the maturity of the cereals.

The attacks of stem rust and several other diseases are most severe when the plants are nearly mature, or when the kernels begin to form. The decreasing temperatures delay maturity at the time when the humidity is increasing. This combination is most favorable to attacks of many cereal diseases. The natural deficiency in humidity in the early fall, as compared with the similar period in the spring, is remedied by the application of irrigation water. This method in invariably effective so far as concerns the creating of favorable conditions for disease attack.

DISEASE EPIDEMICS OF CEREALS INDUCED BY JULY SOWING

The diseases found to be most readily influenced by the method of fall sowing are those which depend upon the air for transfer of spores in natural

infestation. Such diseases must have optimum moisture and temperature conditions. These conditions can be induced at will by means of irrigation and early July sowing. In late October and November, the plants have reached the shooting and heading stages, when they are most susceptible to attacks of wind-borne diseases. At the same time, the optimum conditions for disease development, so far as moisture and temperature are concerned, have arrived. This period of optimum conditions for diseases apparently covers about five weeks after the repressive summer heat has passed and before the exclusively low temperatures of winter have set in.

The effect of the fall weather on maturing cereals is exactly the reverse of that of the spring conditions. All winter-sown varieties tend to mature in a given short period in the spring, but the same varieties sown in July spread the period of their maturity over a much longer time. Some of them fail to mature. By this means the early plants can be separated with ease from the later-maturing ones in families still heterozygous for this maturity factor.

For the past three years, careful and repeated observations on July-sown cereals have shown that the following cereal diseases may be induced in epidemic severity: on wheat, stem rust (*Puccinia graminis*), leaf rust (*Puccinia triticina*), mildew (*Erysiphe graminis*), and spot blotch (*Septoria tritici*); on barley, stem rust (*Puccinia graminis*), leaf rust (*Puccinia anomala*), scald (*Rhynchosporium secalis*), net blotch (*Helminthosporium teres*), spot blotch (*Helminthosporium sativum*), and mildew (*Erysiphe graminis*); and on oats, stem rust (*Puccinia graminis avenae*), crown rust (*Puccinia coronata*), and mildew (*Erysiphe graminis*).

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A POSSIBLE ALTERNATE STAGE OF PUCCINIASTRUM MYRTILLI (SCHUM.) ARTH.

J. S. Boyce

In the East it has been shown that the alternate stage of *Pucciniastrum myrtilli* on various Ericaceous hosts is a *Peridermium* on eastern hemlock, *Tsuga canadensis* L. (1, p. 719; 2, pp. 237–238; 3, p. 27; 4, pp. 33–35). In western Oregon and Washington this rust is reported as very abundant on *Vaccinium macrophyllum* (Hook.) Piper, common on *V. ovalifolium* Smith and *V. caespitosum* Michx., and is rarely encountered on other species such as *V. parvifolium* Smith and *Oxycoccus macrocarpus* (Ait.) Pursh. The writer has found it in this region on only the two species first named. *V. macrophyllum* normally grows at the higher elevations in the mountains, being particularly abundant in the Cascades, where it is commonly and intimately associated with mountain hemlock (*T. mertensiana* (Bong.) Sarg.) and to a lesser extent with western hemlock (*T. heterophylla* (Raf.) Sarg.). While more frequently encountered at lower levels, *V. ovalifolium* is sometimes found with *V. macrophyllum*. In spite of the abundance of *P. myrtilli* on these two huckleberries, no *Peridermium* has ever been found on the hemlocks. The only hemlock rust known in the region is *Caeoma dubium* Ludwig, which is confined to western hemlock. The distribution of this hemlock needle rust as observed over a number of seasons does not indicate a possible connection with *P. myrtilli*.

On May 28, 1924, in Clackamas Co., Oregon, at Government Camp, which is located on the south slope of Mt Hood at an elevation of approximately 4,000 feet, a *Peridermium* (F. P. No. 40402, B. No. 1337)[1] of the cylindrical, columnar type was extremely abundant on the 1923 or previous season's needles of Pacific fir [*Abies amabilis* (Loud.) Forbes]. The current season's, or 1924, needles had not appeared, the buds not yet being open. There was no rust found on western hemlock, mountain hemlock, or noble fir (*A. nobilis* Lind.). *Peridermium ornamentale* Arth. was sparingly present on the needles of alpine fir (*A. lasiocarpa* (Hook.) Nutt.). *Peridermium sp.*, by which the *Peridermium* on silver fir will be designated in this paper, was so abundant that it seemed there should be some clue to

[1] F.P. = Herbarium of the Office of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture. B. = Herbarium of J. S. Boyce. The collections cited in this paper have been examined by Dr. H. S. Jackson.

the alternate host. This was found in the fact that infected silver firs were invariably close to *V. macrophyllum* and that where the huckleberry did not occur there was no *Peridermium sp.*

Further study in this locality was made on July 18. *Peridermium sp.* on silver fir was abundant, although most of the peridia had ruptured and discharged the aeciospores. In the immediate proximity of the infected firs, *P. myrtilli* in the uredinial stage was abundant on *V. macrophyllum* (F. P. No. 40351, B. No. 1341). Owing to absence from the region during the entire growing season of 1925 no observations were made that year, but on May 2, 1926, *Peridermium sp.* was found to be very scarce, a search of two hours yielding only four infected needles. In 1927 conditions were different. While *Peridermium sp.* was not nearly so generally abundant as in 1924, on June 19 it was quite common locally, being closely restricted to the moister localities along the streams and the fringes of small swamps. On August 21 there was a light infection of *P. myrtilli* on the shade forms only of *V. macrophyllum* and *V. ovalifolium,* always within a few feet of heavily infected silver fir.

It has been found that the age of the needles on which the aecia develop is of diagnostic value in the needle rusts of balsam firs. This *Peridermium* is one of the three in the region which appear on needles one year old. In other words, in 1927 the aecia appear on 1926 needles only. One of these, *Uredinopsis macrosperma* (Cooke) Magn., is found commonly on lowland white fir (*A. grandis* Lind.), and its white aecia, which appear in the spring or early summer, are in decided contrast to the orange-yellow aecia of *Peridermium sp. Uredinopsis macrosperma* so far has not been found associated with *Peridermium sp.,* but *P. ornamentale* Arth. has been observed each season at Government Camp, confined, however, to alpine fir. While microscopically there is no significant difference between *P. ornamentale* and *Peridermium sp.,* the former produces stout orange-yellow aecia, very much flattened laterally. Furthermore, the two species do not accord in their seasonal frequence. In 1924 when *Peridermium sp.* was so generally abundant, *P. ornamentale* was very scarce. On the other hand, in 1926 when *Peridermium sp.* was so exceptionally scarce, *P. ornamentale* was common, while in 1927 when *Peridermium sp.* was locally abundant, *P. ornamentale* was generally so. From the foregoing it seems that *Peridermium sp.* on silver fir is different from any other *Peridermium* in this region appearing on needles of the same age.

Inoculation experiments have so far failed. On May 2, 1926, at Government Camp, seven plants of *V. macrophyllum* were dug from a spot at an elevation of 4,000 feet where there had been considerable infection of *P. myrtilli* in 1924. These were replanted the same day in a garden at Portland, Oregon, at an elevation of 100 feet. Only two of the plants survived

during the season, both remaining free from rust. The only silver firs in Portland are scattered ornamentals, because the species does not grow naturally at such a low altitude. No silver firs are known in the general neighborhood of this garden, and the nearest forest trees probably are 40 miles away. During the winter another plant died. On June 10, 1927, the one survivor was inoculated with *Peridermium sp.*, brought from Government Camp, by dusting spores from infected sprays on the thoroughly moistened huckleberry leaves. Again on June 19 the operation was repeated with fresh inoculum from the same locality, and in addition a large spray of heavily infected needles was left resting on top of the huckleberry plant and several smaller sprays were left on the ground at its base. The succeeding days were cloudy with intermittent rainfall. No infection resulted from these inoculations, but this was expected since the huckleberry in the garden at an elevation of 100 feet was at least a month older in development than those growing naturally at 4,000 feet at Government Camp. The leaves of the former were dark green in color, tough and leathery, while those of the latter at the same time were light yellow-green in color, soft and succulent. Then, too, the usual lack of vigor in a plant removed to a new environment where soil conditions, particularly acidity, were quite different from its original habitat very probably influenced its reaction to inoculation.

From the foregoing discussion it seems reasonable to conclude that *Peridermium sp.* on needles of the previous season of silver fir on Mt. Hood differs from other Peridermia in this region and that it may be the aecial stage of *Pucciniastrum myrtilli*. This latter possibility is strong enough to warrant inoculation experiments.

This paper is not complete and is based on circumstantial evidence, which in such a complicated group as the needle rusts of balsam firs may be completely misleading, but the information is given with the hope that it may help some future investigator, since the writer can not continue the work.

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